

SHORT REPORT

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## Increased Serum Cu/Zn SOD in Individuals with Clinical Depression Normalizes After Zinc and Anti-oxidant Therapy

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### Abstract

**Aim:** To assess serum Cu/Zn SOD (Superoxide Dismutase) concentration in individuals with clinical depression.

**Subjects and methods:** Serum from 36 individuals diagnosed with clinical depression and 18 healthy controls were tested for Cu/Zn SOD serum concentration using ELISAs.

**Results:** Serum Cu/Zn SOD levels of depressed individuals (both with and without secondary anxiety) were significantly higher than age and gender similar controls. We also found that, post anti-oxidant therapy, Cu/Zn SOD levels normalized to the level of normal healthy controls.

**Discussion:** These results suggest an association between Cu/Zn SOD serum levels and clinical depression.

**Keywords:** depression, Cu/Zn SOD, super oxide dismutase, oxidative stress

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## Introduction

Clinical depression is a considerable public health problem.<sup>1</sup> Approximately 32 to 35 million adults in the United States have experienced depression at some point in their life and approximately 13 million adults have experienced depression within the past year.<sup>2</sup> Clinical depression is also a considerable medical problem as those with major depressive disorder (MDD) are at increased risk for serious medical illness, including cardiovascular disease,<sup>3–5</sup> diabetes,<sup>6–8</sup> cancer,<sup>9–11</sup> and stroke.<sup>12</sup> This risk is often independent of traditional risk factors, suggesting that depression may function as a causal factor in the pathogenesis of multiple diseases. To understand the pathophysiological effects of depression, we examined one of the biologic mechanisms common to multiple diseases: oxidative damage to DNA.

Oxidative damage results from biochemical interactions between reactive oxygen species (ROS) and target biomolecules. ROS can damage nucleic acids, lipids, and proteins, and figures prominently in the etiology and progression of numerous cancers<sup>13–15</sup> as well as coronary and carotid atherosclerosis.<sup>16–19</sup>

Oxidative stress, occurring as a consequence of imbalance between the formation of free oxygen radicals and inactivation of these species by antioxidant defense system, is capable of causing damage to various cellular and extracellular constituents. The deleterious effects of increased oxidative stress are termed oxidative damage. These effects generally appear after exposure to a relatively high concentration of reactive oxygen species (ROS) and/or a decrease in antioxidant (AO) defense system against ROS.<sup>20</sup>

*In vivo*, oxygen radicals are produced as byproducts of normal oxidative metabolism.<sup>21</sup> Hence, activated cells with increased metabolism produce more oxygen radicals. It has long been known that control of the intracellular redox environment is vital for proper cellular function. To protect themselves from the constant oxidative challenge, cells have developed defense mechanisms that ensure a proper balance between pro- and antioxidant molecules.<sup>22</sup>

Cu/Zn superoxide dismutase (SOD-1) is a key enzyme in the dismutation of superoxide radicals resulting from cellular oxidative metabolism, converting them into hydrogen peroxide,<sup>23</sup> and, as a result, serves a key antioxidant role. In fact, mice lacking SOD die several days after birth, amidst massive

oxidative stress.<sup>24</sup> Copper, and zinc participate in SOD enzymatic mechanisms that protect against free radicals and therefore serve an important adjunct role in oxidative balance.<sup>25</sup>

Our study was designed to determine Cu/Zn SOD serum levels in clinically depressed individuals and test the hypothesis that this oxidative stress marker is increased in depressed individuals. This oxidative imbalance may lead to depressive symptoms.

## Materials and Methods

### ELISA to measure serum Cu/Zn SOD (bender systems)

All reagents and specimens were equilibrated to room temperature before the assay was performed. A 1:51 dilution of the patient samples was prepared by mixing 10  $\mu$ l of the patient's sera with 0.5 ml of Serum Diluent. One hundred microliters of calibrators (0.08–2.5 ng/ml Cu/Zn SOD), serum diluent alone, and diluted patient samples were added to the appropriate microwells of a microculture plate (each well contained affinity purified polyclonal IgG to Cu/Zn SOD). Wells were incubated for 60 minutes ( $\pm$  5 min) at room temperature, then washed 4 $\times$  with wash buffer. One hundred microliters of pre-diluter anti-human Cu/Zn SOD IgG conjugated with HRP was added to all microwells, incubated for 30 minutes ( $\pm$  5 min) at room temperature, then wash 4 $\times$  with wash buffer. One hundred microliters of enzyme substrate was added to each microwell. After approximately 30 minutes at room temperature, the reaction was stopped by adding 50  $\mu$ l of 1M sulfuric acid, then the wells were read at 450 nm with an ELISA reader (BioRad Laboratories, Inc., Hercules, CA, USA).

## Subjects

### Experimental and controls

Serum from individuals with diagnosed clinical depression (n = 26; 10 male; mean age 35.2 years) and controls (n = 19; 5 male; mean age 41.7 years) was obtained from patients presenting at the Health Research Institute/Pfeiffer Treatment Center Warrenville, IL\*. Patients were chosen for this study by presenting to the Pfeiffer Treatment Center for depression and agreeing to be involved in research at the Center. Most of these

\* The Pfeiffer Treatment Center is a comprehensive research and treatment center, specializing in the care of with neurological disorders, including Depression.

individuals were diagnosed using The Hamilton Rating Scale for Depression before presenting at the Pfeiffer Treatment Center. Healthy controls included individuals with no documented mental illness, recruited from the community and agreeing to the research.

The sample size was determined by the number of depression patients and controls available at the time of the study.

Cases and controls were age and gender similar (see above). Smokers and individuals taking medications which might affect oxidative stress, such as steroids, statins and indole-derived drugs, were eliminated from the study.

### Anti-oxidant Therapy

Individuals in this study who presented to the Pfeiffer Treatment Center with depression were tested for anti-oxidant levels. Based on deficiencies, they were then prescribed the appropriate dosage of anti-oxidant. Pre-therapy patients represent those who were tested when they first presented and were not previously taking any anti-oxidants. Post-Therapy patients received anti-oxidant therapy, including zinc supplementation if necessary, for a minimum of 8 weeks.

### Serum/Plasma

All experimental and control serums were drawn using venipuncture then treated in an identical fashion—frozen at  $-70\text{ C}$  immediately after collection and cell/serum separation, then stored at  $-70\text{ C}$  until thawed for use in ELISAs.

### Statistics

Inferential statistics were derived from unpaired t-tests.

### Results

Serum from 36 individuals diagnosed with clinical depression and 18 age and gender matched controls was tested for Cu/Zn SOD plasma concentration using an ELISA (described above). Each assay was repeated two or more times, with multiple wells for each serum in each assay. Results of a typical assay with assay controls and depressed and control individuals are shown in Figures 1 and 2, respectively.

Serum Cu/Zn SOD levels of depressed individuals were significantly higher than all non-depressed controls ( $P < 0.0025$ ) (Table 1). Although Cu/Zn SOD levels were higher in depressed individuals with secondary anxiety, when compared to depressed individuals without anxiety, this difference was not significant.

Medical records were available for 28 of the 36 depressed individuals in this study. Cu/Zn SOD levels in 18 of these same individuals after anti-oxidant therapy was significantly lower than 10 who presented with depression for the first time (before therapy) ( $P = 0.037$ ) (Table 2) and Cu/Zn SOD levels normalized post therapy when compared to controls ( $P = 0.305$ ) (Table 4).

Perceived severity of depression (scale of 0–5 (5 being the most severe depression)) was asked of 21 of the 36 depressed individuals in this study. There was a significantly lower level of perceived depression post anti-oxidant therapy ( $P = 0.0031$ ) (Table 3).

### Discussion

Oxidative stress has been implicated in the pathogenesis of a diverse group of disease states,

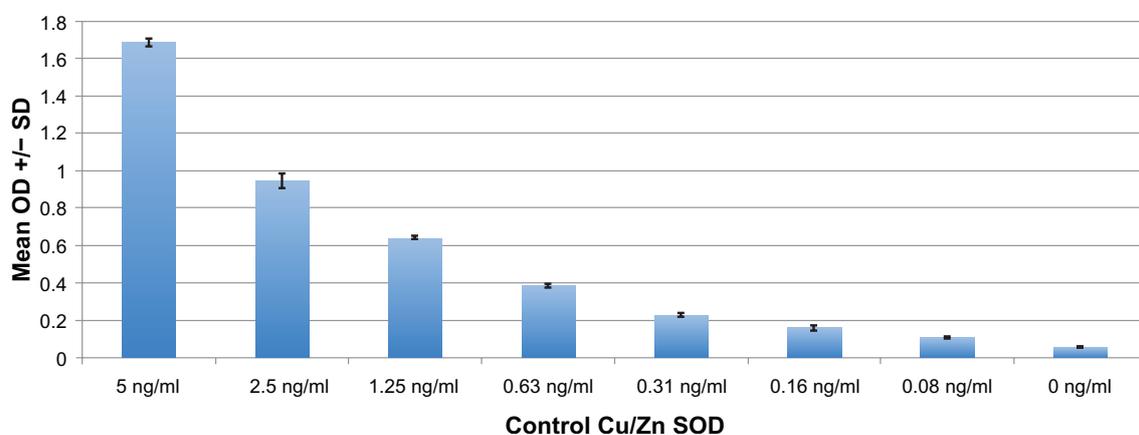
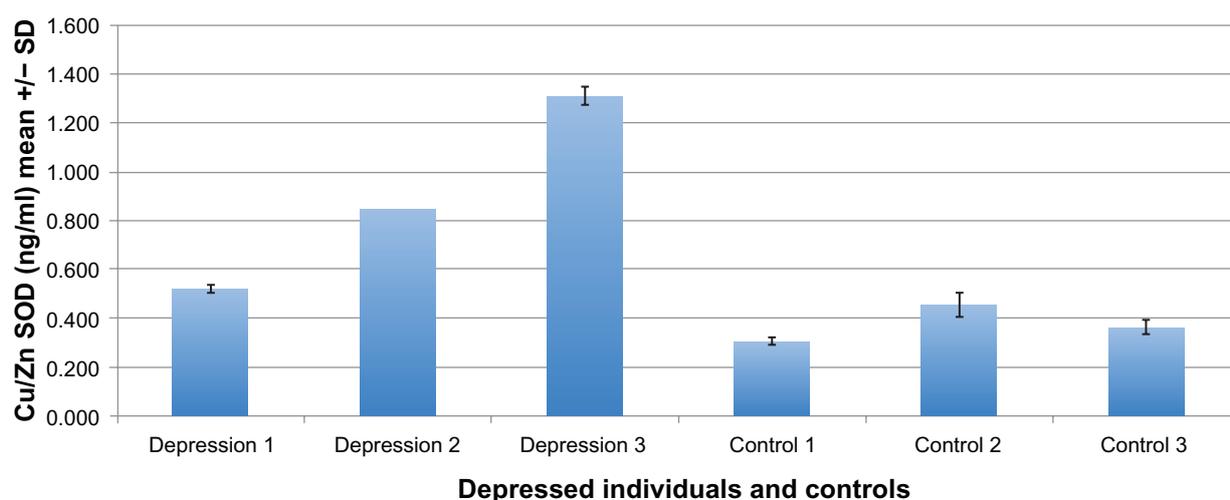


Figure 1. Positive and negative controls of typical ELISA to measure Cu/Zn SOD plasma levels.



**Figure 2.** In a typical ELISA, plasma from 3 individuals diagnosed with clinical depression and three normal (neuro-typical) age/gender matched individuals was tested for the Cu/Zn SOD concentration. Cu/Zn SOD concentration is significantly higher in the depressed individuals.

and, because the brain has comparatively greater vulnerability to oxidative damage, may be a common pathogenic mechanism underlying many major psychiatric disorders.

This study represents an attempt to assess levels of the oxidative damage expressed by Cu/Zn SOD levels in the plasma of clinically depressed, but otherwise healthy adults. As our results demonstrate, compared with matched control subjects, those with clinical depression show significantly higher serum levels of Cu/Zn SOD.

Other studies have shown positive correlations between levels of 8-OHdG,<sup>40</sup> negative mood, as measured by Profile of Mood States,<sup>26</sup> perceived workload, psychological distress and the perceived impossibility of alleviating distress,<sup>27</sup> and severe depression as measured by the General Health Questionnaire.<sup>28</sup>

Clinical depression associated with increased oxidative damage may represent a common pathophysiological mechanism, whereby these patients become vulnerable to multiple comorbid medical illnesses.<sup>29</sup>

**Table 1.** Cu/Zn SOD plasma levels in depressed individuals are significantly higher than in normal controls.

	Depression	Normal controls
Mean	0.695	0.326
SD	0.479	0.149
SEM	0.080	0.035
N	36	18

**Note:** The two-tailed *P* value equals 0.0025. *t* = 3.1769; *df* = 52; standard error of difference = 0.116.

Depression is associated with an activation of innate immune responses,<sup>30</sup> inflammation<sup>31</sup> and heightened vulnerability to latent infection.<sup>32,33</sup> Activated phagocytes are significant sources of ROS and produce superoxide, hydrogen peroxide, nitric oxide, and peroxynitrite as part of the cytotoxic host response against invading pathogens.<sup>34</sup>

Depression may contribute to oxidative damage through an increase in the production of reactive oxygen species (ROS). ROS can be antimicrobial; they damage lipid membranes and protein structures, thus destroying antigen-bearing cells. Oxidative damage is not limited to microbial targets, however, and extensive host tissue damage may result.<sup>35</sup> ROS from activated phagocytes can damage DNA bases<sup>36</sup> and induce strand breaks in neighboring cells,<sup>37</sup> leading some to argue that the hydroxyl radicals and peroxynitrite formed during inflammation are the greatest contributors to the oxidation of DNA.<sup>38</sup>

**Table 2.** Individuals with clinical depression had significantly lower serum Cu/Zn SOD concentration (ng/ml) after antioxidant therapy.

	Cu/Zn SOD before therapy	Cu/Zn SOD after therapy
Mean	0.751	0.437
SD	0.331	0.412
SEM	0.078	0.130
N	18	10

**Note:** The two-tailed *P* value equals 0.0368. *t* = 2.2018; *df* = 26; standard error of difference = 0.142.

**Table 3.** When individuals with depression were asked to assess their levels of depression (0–5; 5 being the most severe), depression severity was significantly lower after therapy.

	Depression severity no therapy	Depression severity after therapy
Mean	4.154	2.688
SD	0.718	1.28
SEM	0.199	0.453
N	13	8

**Note:** The two-tailed *P* value equals 0.0031. *t* = 3.3846; *df* = 19; standard error of difference = 0.433.

Depression may not increase production or exposure to ROS, but rather decrease repair of damaged DNA. There is some evidence that repair of x-ray-damaged DNA is slower among highly distressed psychiatric inpatients, the majority of whom were hospitalized for depression.<sup>39</sup>

Recent research demonstrates that, in a chronic mild stress (CMS) rat model, which induces some symptoms of a major depressive episode in humans, superoxide production was increased in all brain structures analyzed.<sup>41</sup> Despite the fact that Cu/Zn SOD is a very non specific marker of oxidative stress, the levels of which basically signify the overall redox balance in body, others have found it to be a useful clinical biomarker of oxidative stress.<sup>42,43</sup> Our data suggests that Cu/Zn SOD levels in plasma may reflect this overproduction and may make Cu/Zn SOD a good biomarker for the monitoring of clinical depression.

The clinical relevance of increased oxidative damage, evidenced by clinically depressed individuals, is currently unknown, and future studies should appropriately address this deficit. Our data are consistent with the hypothesis that oxidative damage is a potential common pathophysiological mechanism underlying multiple co-morbid conditions in depressed

**Table 4.** Cu/Zn SOD levels normalized in depressed individuals after anti-oxidant therapy and zinc supplementation.

	Cu/Zn SOD after therapy	Cu/Zn SOD controls
Mean	0.437	0.326
SD	0.412	0.149
SEM	0.130	0.035
N	10	18

**Note:** The two-tailed *P* value equals 0.3049. *t* = 1.0466; *df* = 26; standard error of difference = 0.107.

people. Future studies should include measurement of multiple oxidative damage markers to different macromolecules, associated dietary deficiencies associated with these markers, associated severity of depression, and should address whether depression remission remediates underlying oxidative damage.

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