Correlation of Serum Cu and Zn with Some Cytokines in Major Depressive Disorder

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Abstract

There is an association of major depressive disorder with different changes in the immune functioning which associate with activation of inflammatory processes. Trace elements such as copper (Cu) and zinc (Zn) have many physiological functions. In the present study, serum levels of Cu, Zn, inflammatory (IL-6), and anti-inflammatory (IL-10) cytokines have been measured in MDD patients and compared with the apparently healthy group. The correlation between the measured trace elements and IL-6 and IL-10 is another purpose of the study to find out the trace elements effect on the secretion of the interleukins in MDD patients. The study involved 60 pretreatment males with MDD and 30 apparently healthy as controls (age matched). The results indicated that there was significantly increase (p<0.05) in IL-6 and IL-10 in MDD males in comparing with controls. There was no significance difference between the studied groups for each of Cu and Zn and the ratio of the two elements. The correlation results indicate that there was a positive correlation between both Cu and Cu/Zn ratio with IL-6 only. It can be concluded that there is a correlation between both serum Cu level and Cu/Zn ratio with proinflammatory marker IL-6, which indicates that trace element, is correlated with the magnitude of the inflammatory response.

Keywords: *Copper; Zinc; IL-6, IL-10, Major Depressive Disorder.*

Introduction

Depressive disorder is a health problem that weakens psychosocial and occupational functioning and is correlated with significant morbidity and mortality [1]. By WHO, depressive disorder is now the fourth of disability leading cause in the world, and it has been expected that this disease will be as the second leading cause of disability at 2020 [2] and it is believed that by year 2030 the lost in disability of life related to depression more than other illness [3].

Major depressive disorder (MDD) is a neuropsychiatric syndrome characterized by low mood and anhedonia, along with somatic and cognitive disturbances [4].It is presumed that MDD is correlated with changes in the functioning of immunity (associate with inflammatory processes activation) [5]. Patients with MDD show changes in immunological markers including rises in inflammatory markers (cytokines) like IL-6 and IL-1ß activity and inflammation [6]. Changed functioning of the immune system is concerned as a mechanism which might contribute to medical morbidity of MDD including risk of infectious disease [7]. Interaction between immune and nervous systems is essentially related pathophysiology of depression [8]. The role of inflammation in the pathophysiology of MDD has been increasingly predictable and is now designated as the inflammatory or "cytokine hypothesis" of depression [9].

There are also a plenty of evidence that increased pro-inflammatory cytokine levels and an increased immune response are associated with depression diagnosis, symptomatology, and severity [10]. Major depression is found to be associated with dysregulation of immune biomarkers, like the increase in IL-1ß, IL-6, IL-12, IL-2, soluble IL-2R, and IL-1Ra which have been considered as an imbalance between pro- and anti-inflammatory cytokines in major depression. [11]. Trace elements, like copper (Cu) and zinc (Zn) have been examined for

many decades [12]. They have an important role in various biological processes in humans, animals and plants that are essential for life and normal development [13, 14]. The concentration or metabolic disturbance of the two elements can cause development of serious metabolic disorders such as psychiatric disorders [15].

Zn is firmly controlled in the brain mainly located within glutamatergic neurons [16]. Zn inadequacy is associated with neuropsychiatric manifestations that can exist as changed behavior and cognition, reduced learn ability, and depression [17]. Cu is an essential trace element, which has been found to be an important constituent of vital Cu-dependent enzymes acts as a cofactor of many redox enzymes [18]. This element is also essential to the appropriate development and CNS functioning; low Cu concentration may cause inadequate development, whereas high concentration could be harmful [19].

Limited data proposes the potential importance of Cu in the development of neuropsychiatric disorders, including depression [20] depending on the fact that Cu is an important component of some enzymes required for the turnover of catecholamine's, which disturbances may cause depression development [21].The aim of the present study is estimation of the influence of Cu and Zn on the level of IL-6, and IL-10 levels in un-treated males with MDD.

Subjects and Methods

The study depending on case-control design of 60 new case male patients with MDD aged 25-65 year compared with 30 apparently healthy as a control group. Samples were collected from the "Psychiatry Unit at Al-Hakeem General Hospital in Najaf Governorate-Iraq". In addition to patients samples got from a private psychiatric clinics by a senior psychiatrist. Full medical history were obtained for all patients and any

existing systemic disease that could interfere with the detected parameters, particularly DM, liver and kidney diseases, was omitted from the study. Any treated MDD case, addict or post operation persons that may affect the parameters were also excluded. CRP test was evaluated and it was negative in all of the samples (CRP<4 mg/L). The diagnosing of the patients was by the psychiatrists depending on DSM-IV criteria.

Measurements

Five milliliters of venous blood samples were drown by utilizing disposable needle and plastic syringes from each patient and control. Samples with hemolysis were discarded. The blood was kept at room temperature for 15 minutes for clotting, centrifuged 3000 xg for 10 minutes. Serum was stored in freeze until analyzed. Serums Cu, Zn were measured by Flam Atomic Absorption, while the two interleukins were measured by ELISA technique.

Statistical Analysis

The statistical analysis was achieved by SPSS Version 25 (2017) "IBM-USA", and the figures constructed by Excel program of Microsoft Office 2016. The results were expressed as (mean ± standard deviation). Pooled t-test has been used for the comparison between the patients and control groups and among subgroups in the measured parameters. Pearson's correlation coefficients (r) were calculated to estimate the correlation between parameters. The difference between groups is considered as statistically different when $p<0.05$.

Results

Demographic and Clinical Characteristics

The demographic and clinical characteristics of MDD patients and control groups are existing as observational data in Table 1. They exhibited almost the same age and gender.

Table1: Demographic and clinical characteristics of patients and controls

Parameter	Patients	Control
Gender: male(female)	60(0)	30(0)
Age, year	25-65	24-68
Demographic area: Urban (Rural)	45(15)	25(5)
Education: Learned (illiterate)	50(10)	28(2)
Employment: Employed (Not Employed)	40(20)	26(4)
Marital status: married (single)	40 (20)	14(16)
Smoking (not smoking)	25 (35)	0(30)
Treatment: Treated (not-Treated)	0(60)	0(0)

These data indicated that most of the MDD group was wedded. The smokers were less than half of the patient's number. In order to eliminate drugs effect the measured parameters levels**,** all cases were newly diagnosed (pretreatment).

Comparison between MDD Patients and Control Group

The levels of serum Cu, Zn, IL-6 and IL-10 in the patients were illustrated in Table 2. The result indicate that both cytokines level were significantly elevated in MDD patients more than controls, while trace elements didn't show any significant difference between the two groups.

Table 2: Comparison between MDD patients and control group

Parameter	Patients	Control	p-value
Cu (ppm)	0.85 ± 0.59	0.76 ± 0.21	NS
Zn (ppm)	0.50 ± 0.23	0.52 ± 0.15	NS
Cu/Zn	2.24 ± 2.09	1.77 ± 1.32	NS
IL-6 pg/ml	13.39±3.04	11.44±2.21	0.046
IL-10 pg/ml	9.52±2.24	8.03±1.4	0.01

Correlation among Parameters in MDD Group

The result in Table-3 showed that there was no relation between the studied parameters.

Table 3: Correlation among parameters in MDD group

		IL-6	IL-10
Cu	r	0.469*	-0.050
	p	0.004	0.646
Zn	r	0.044	0.040
	p	0.800	0.718
Cu/Zn	r	0.353*	-0.011
	p	0.035	0.922

The positive correlation between both Cu and Cu/Zn ratio with IL-6 as shown in Figure 1.
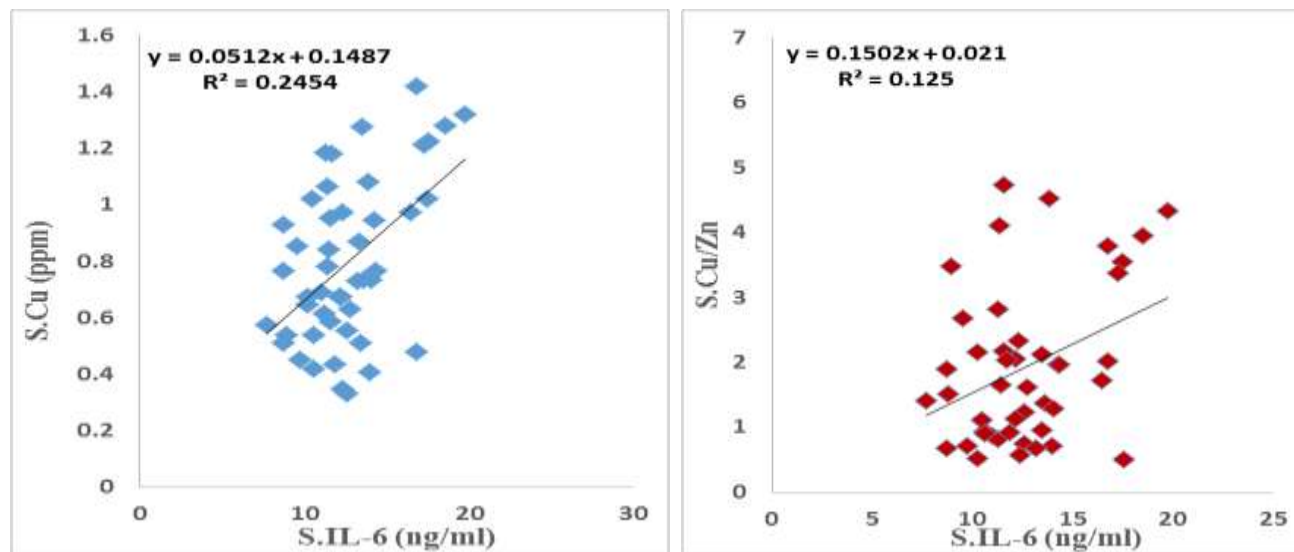

Figure 1: Correlation between IL-6 and both Cu and Cu/Zn ratio

Discussion

The results of the current study was achieved depending on the available cases which was obtained from narrow area, furthermore they must be pretreatment that leads to get low number of cases as shown in Table 1.

Comparison between Patients with MDD and Control Groups

As shown in Table 2, there is a significant increase ($p<0.05$) for IL-6 and IL-10 in MDD in comparison to control group. The obtained results are in line with several studies that have noticed increase inflammation level in depressed individuals in comparing with healthy controls. These results indicate that MDD exhibited immunological response to inflammation. In coincidence with the present study findings, many studies indicate that IL-6 level increase in MDD patients [22-23]. IL-10 is an important cytokines required for the controlling of inflammatory responses. It has the capacity to destroy the pro-inflammatory cytokines production and shows an essential role in the overactive

responses regulation that may leads to auto-inflammatory diseases [24]. There were suggestions from different studies about the relationship between psychiatric disorders and IL-10 production, which are varying with those describing increased [25] and unchanged [26].

The results of current study is in line with thus studies indicating an increase of IL-10 level in MDD in comparison with control group [27], and disagree with other studies that prove a decrease in IL-10 level. From Table 2, the results of MDD patients and control groups comparison show no significant difference between them. The result of present study is in agreement with Styczeń *et al.*, (2016) whom proved that the analysis of variance showed that no significant influence of depressive patients in compare with healthy group regarding to serum Cu concentrations (p>0.05) [28]. In concern to Zn, Porter (2011) study is in line with the present results.

This study reported the means of depressed and control groups to be within normal laboratory reference ranges [29]. Whereas Gezel *et al.*, showed that the mean Zn level old people with depressive disorder was significantly higher than those with non-depressive disorder, but the mean serum Cu level in two groups was the same [30]. Engle-Stone *et al.*, found that Zn level was both significantly less in depressive group and negatively interrelated to the severity of depressive symptoms [31].

Several studies have demonstrated lower Zn levels in patients with depression than in healthy controls [32]. The other important parameter is Zn/Cu ratio, which is essential for appropriate function of human organism. Moreover, it is clinically more important than the detection of either of the two elements alone.

They also suggest that the imbalance in Zn-Cu levels can lead to the progress of many diseases, mainly psychiatric disorders "e.g., depression, postpartum depression, schizophrenia, autism spectrum disorders" [18]. Although Cu and Zn have an important role in functioning of CNS and associate with neuropsychiatric disorders, the results of the present study suggest that the statistically non-significance of both elements between the studied groups reflect low association of these elements with psychiatric disorders.

Correlation between Trace Elements Cu and Zn with Cytokines

The levels of these elements in plasma and tissue are influenced by stress, infection [33], and inflammation [34]. Under such conditions, Zn levels are normally reduced and Cu levels are elevated [35]. The fetal growth regulations critically depend on both Cu and Zn, particularly through the maturation of the nervous system [36]. Mei-Yu *et al.*, (2014) found that serum levels of Cu and IL-6(r=0.393; p<0.05) were positively correlated in the mild-to-moderate depressive individuals.

Those groups had a significantly higher level of depressive symptoms than control group and those reported by the day- and evening-shift nurses [37]. Kurt *et al.*, (2009) studied the relation between IL-6 and Cu level in chronic hepatitis. They indicated that there was no correlation between Zn, Cu and the measured immunological parameters, in patients with chronic hepatitis C and in healthy control subjects [38].

From different studies (animal and clinical studies with adult patients), it was well recognized that inflammatory cytokines disrupt the trace element homeostasis [39]. In addition, the Cu/Zn ratio is changed in certain diseases [40]. Cu/Zn ratio is one of the most common trace-metal imbalances which refer to elevated Cu and depressed Zn. The ratio of Cu to Zn is clinically more important than these trace metals alone [18]. The authors proposed this ratio as an important clinical inflammatory-nutritional biomarker.

Moreover, they suggest the Cu/Zn ratio as a significant predictor of all-cause mortality in people over 70 years of age and presumed that the serum ratio of Cu/Zn concentration is considered to be a marker of inflammation due to its association with elevated IL-6 levels and CRP [41]. The serum or plasma Cu/Zn ratio is amongst those parameters that may be related with reduced ability to maintain or regain homeostasis after a destabilizing event [42].

There was a suggestion that trace-element responses to acute and chronic illness are similar and are related to the magnitude of the inflammatory response [43]. Another possible explanation to the relation between Cu/Zn ratio and IL-6 might gate from the opinion that this ratio could be delicate to

other conditions, such as dietary habits and biological aging and subclinical pathological changes [41].

Conclusions

There is a correlation between both serum Cu level and Cu/Zn ratio with the proinflammatory marker (IL-6) which indicates that trace-element is related to the magnitude of the proinflammatory response in MDD group.

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