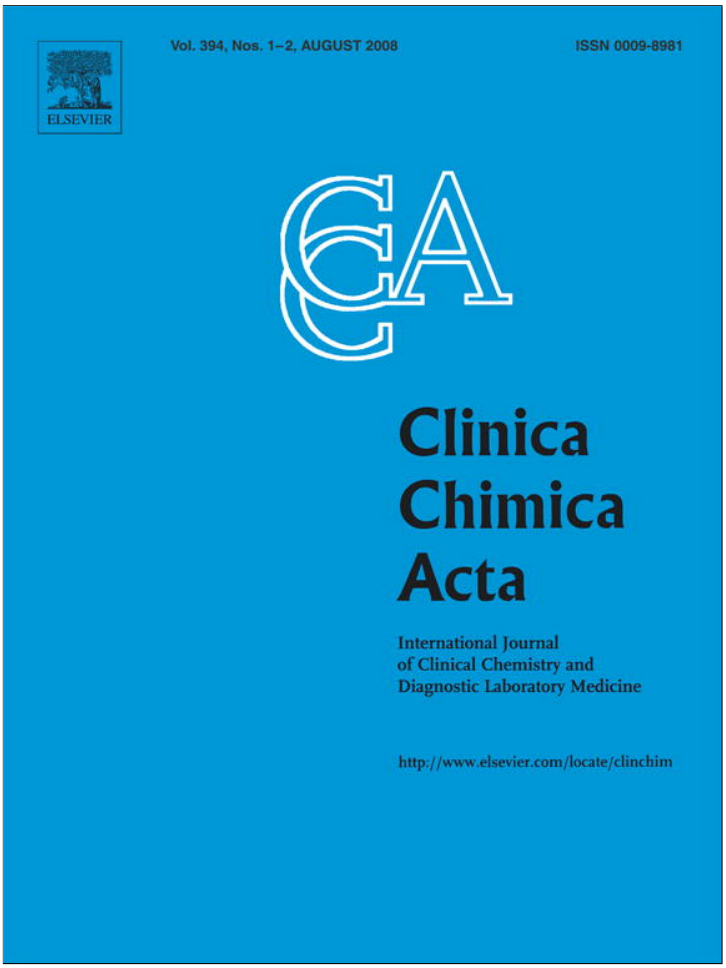

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Assessment of serum macro and trace element homeostasis and the complexity of inter-element relations in bipolar mood disorders

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ABSTRACT

Background: Bipolar disorders are complex neuropsychiatric in nature and are clinically classified as Type I, Type II, and Type V. The etiological factors include environmental–genetic inter-relations. Trace metals play a significant role in neurological disorders. There is very limited information on the role of macro and trace elements in bipolar disorders.
Methods: Trace elements namely Na, K, S, Ca, Mg, P, Cu, Fe, Zn, Mn and Al were analyzed in serum samples of 3 bipolar types: bipolar I, bipolar II and bipolar V with a control group using inductively coupled plasma–atomic emission spectrometry (ICP–AES). The patients were assessed as per the standard diagnostic criteria and classified into the bipolar type I, II hypomanic, II depressives and V.
Results: In bipolar I (mania), Na, K, P, Cu, Al and Mn were increased significantly ($p<0.001$). In bipolar II hypomania, Na, S, Al and Mn were increased significantly ($p<0.02$), while in bipolar II depression, Na, K, Cu and Al were increased ($p<0.001$). In bipolar V, Na, Mg, P, Cu, and Al were increased significantly ($p<0.002$), though S ($p<0.00001$), Fe ($p<0.002$) and Zn ($p<0.004$) were decreased in all 3 bipolar groups.
Conclusions: There is a disturbance in the charge distribution and element–element interdependency in bipolar serum when compared to controls. These results suggest that there is a definite imbalance in macro and trace element homeostasis as evidenced by element inter-relationships in serum samples of bipolar groups when compared to controls.

1. Introduction

Macro and trace elements play a dual role in the biological system through their interaction with biomolecules. They regulate a number of cellular metabolic reactions, while a few of them act as etiological agents in many environmentally induced neurological disorders [1–2]. Elements are required at optimum concentration for proper functioning of the human biological system. Their deficiency would cause various metabolic disorders while increased concentrations are toxic [3]. Elements like Al, Pb, and Cd are highly toxic even in trace amounts and have been reported to be associated with neurological disorders. Other studies have shown that trace elements are involved in neuropsychiatric illness [4]. Toxic metals like Pb and Cd are increased in depressives and schizophrenics but reduced in manic patients [5], and Cu, Zn, and Cs deficiencies are seen in women affected by chronic depression [6–7]. It has been found that the concentrations of Mg and Zn are decreased in serum samples of schizophrenia and dementia patients [8].

Although pivotal biochemical alterations underlying neuropsychiatric disorders are unknown, changes in trace elements play important roles in the bipolar disorder. For instances V an essential element has been implicated as a causative factor for bipolar mood disorder, and elevation of V and Mo has been reported in serum samples of bipolar mood disorder [9] and [10]. Br is significantly increased in serum and hair of bipolar patients on Li treatment [11]. A rise in Al and Br concentrations in the serum samples of Li-treated patients has also been reported [12–13]. Also Li is reported to alter the concentrations of elements such as Ca and Mg in serum samples during treatment [14].

Apart from these data we have limited information on the concentrations of trace elements in serum of individuals with bipolar mood disorders [10]. Moreover, there are no inter-elemental complexity studies pertaining to trace elemental concentrations in serum samples of 3 types of bipolar disorder. Though the above-mentioned studies have focused on the individual elements, no attempt has been

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made to understand inter-elemental relationship and trace elemental homeostasis in bipolar disorder. We examine the concentrations of Na, K, S, P, Fe, Mn, Ca, Mg, Zn, Cu and Al in serum samples of bipolar patients types I, II, V and compare them with controls, and also to understand the element homeostasis through element-to-element inter-relationship in bipolar disorder.

2. Materials and methods

2.1. Patients

The concentrations of eleven elements in serum samples of bipolar I (n=40), bipolar II hypomania (n=25), bipolar II depression (n=27) and bipolar V depression (n=25) were assessed in comparison with a control group (n=25). Blood samples of 3 types of bipolar disorder patients were collected from the Department of Psychiatry, JSS Medical College hospital, Mysore, India. The bipolar disorders were classified as bipolar I (mania), bipolar II hypomania, bipolar II depressives and bipolar V. Blood samples of those patients meeting the diagnostic criteria of manic episode, hypomania, depression of bipolar type II and V were collected. Blood was drawn before medication. The age and sex of the patients and the controls are shown in Table 1.

2.2. Clinical diagnostic criteria applied for bipolar disorder groups

The bipolar patients were clinically assessed and diagnosed according to the D.S.M. IV criteria [15] and Young and Klerman [16]. The essential feature of bipolar I disorder is a clinical course that is characterized by the occurrence of one or more manic episodes with mixed or major depressive episodes in the past. Criteria used for manic episode are patients with distinct period of abnormality and persistently increased, expansive, or irritable mood, lasting at least 1 week (or any duration if hospitalization is necessary). During the period of mood disturbance, ≥3 of the following symptoms have persisted (4 if the mood is only irritable) and have been present to a significant degree: they are a) inflated self-esteem or grandiosity. b) decreased need for sleep (e.g., feels rested after only 3 h of sleep), c) more talkative than usual or pressure to keep talking, d) flight of ideas or subjective experience that thoughts are racing, e) distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli), f) increase in goal-directed activity (either socially, at work or school, or sexually) or psychomotor agitation and g) excessive involvement in pleasurable activities that have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments).

The essential feature of bipolar II Disorder is a clinical course that is characterized by the occurrence of one or more major depressive episodes accompanied by at least one hypomanic episode and non occurrence of manic episode. The bipolar II patients were divided depending on whether they were suffering hypomania, or from depression. A distinct period of persistently increased, expansive, or irritable mood, lasting throughout at least 4 days, that is clearly different from the usual non-depressed mood. During the period of hypomania, ≥3 of the following symptoms have persisted (4 if the mood is only irritable) and have been present to a significant degree: they are a) inflated self-esteem or grandiosity, b) decreased need for sleep (e.g., feels rested after only 3 h of sleep), c) more talkative than usual or pressure to keep talking, d) flight of ideas or subjective experience that thoughts are racing, e) distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli), f) increase in goal-directed activity (either socially, at work or school, or sexually) or psychomotor agitation, g) excessive involvement in pleasurable activities that have a high potential for painful, consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments). The episode is associated with an unequivocal change in functioning that is uncharacteristic of the person when not symptomatic. The disturbance in mood and the change in functioning are observable by others. The hypomanic episode is not severe enough to cause marked impairment in social or occupational functioning, or to necessitate hospitalization, and there are no psychotic features. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication, or other treatment) or a general medical condition (e.g., hyperthyroidism).

During the period of depression ≥5 of the following symptoms have persisted the same 2 week period and represent a change from previous functioning and at least 1 of the symptoms is either depressed mood or loss of interest or pleasure: they are a) depressed mood most of the day, nearly every day, as indicated by either subjective report or observation made by others, b) markedly diminished interest or pleasure in all or all most all, activities most of the day, nearly every day, c) significant weight loss when not dieting or weight gain or decrease or increase in appetite nearly everyday, d) insomnia or hypersomnia nearly everyday, e) psychomotor agitation or retardation nearly every day, f) fatigue or loss of energy nearly everyday, g) feeling of worthlessness or excessive or inappropriate guilt nearly everyday, h) diminished ability to think or concentrate or indecisiveness nearly every day, i) recurrent thought of death and suicidal ideation without a specific plan or a suicidal attempt or a specific plan for committing suicide.

The essential feature of bipolar V disorder is characterized by the occurrence of one or more episodes of depression in a person with a significant family history of bipolar disorder are a well recognized condition [16].

Table 1
Age (shown in range and as mean with standard deviation) and gender of the bipolar patients and controls

	Bipolar I	Bipolar II hypomanic	Bipolar II depressives	Bipolar V depressives	Controls
Number	40	25	25	25	25
Age					
Mean±SD	29.5±6.9	27.3±5.8	31.7±7.6	33.9±8.6	27±8.1
Range	22.6–36.4	21.5-33.1	24–39	24–42	21.9–35.1
Gender					
Men	27 (67.5%)	18 (72%)	12 (48%)	15 (60%)	17(68%)
Women	13 (32.5%)	7 (38%)	13 (52%)	10 (40%)	8 (32%)

Table 2
Wavelengths used and the detection limits of the elements analyzed

Element	Wavelength (nm)	Detection limit*	
		μg/mL	μmol/mL
Na	588.995	0.03	0.00130
K	766.49	0.06	0.00153
S	182.98	0.05	0.00156
P	213.618	0.05	0.00162
Ca	393.366	0.002	0.00005
Mg	279.806	0.001	0.00004
Cu	224.7	0.002	0.00003
Zn	213.856	0.002	0.00003
Fe	259.94	0.005	0.00009
Al	396.152	0.002	0.00007
Mn	257.61	0.001	0.00002
Si	251.611	0.08	0.00285

*Detection limit (μg/mL) for each element was calculated by running a multi-element standard solution containing 500 ng/mL of each of the above-cited elements.

2.3. Control groups

Individuals with no known psychiatric problems served as the control group. The control group comprised 25 “healthy” volunteers having neither significant medical illness nor under medications for at least 3 months' duration at the time of blood collection. Both controls and bipolar group were assessed based on DSM IV criteria [15].

2.4. Exclusion criteria adopted

The following exclusion criteria were applied to the bipolar patients and controls [17] and [18]: a) ethanol intake >80 g/day in the last 6 months, b) history of chronic hepatopathy or disease causing malabsorption, c) history of severe systemic disease, d) a typical dietary habits (diets constituted exclusively by one type of food stuff, such as vegetables, fruits, meat, or others, special diets because of religious reason etc., e) previous blood transfusions, anemia and polycythemia, f) intake of supplements of Fe, Cu, Al, Zn or chelating agents, g) acute infectious disorders, traumatisms or surgery in the last 6 months, and h) hemolytic anemia.

2.5. Ethical issues

Ethical approval for collecting blood samples of bipolar patients and controls were obtained from the Research Ethical Committee of J.S.S. Medical College Hospital, Mysore, India. A written consent was obtained from the patients/guardians prior to the collection of blood samples.

2.6. Precaution to avoid cross contamination during sample collection and storage

Venous blood sample was collected from each bipolar patient/control and serum was separated by centrifugation. Blood collection was done and the serum was separated in a dust-free room. The serum was frozen at −20 °C and protected from exposure to light until analysis. All the tubes used were polypropylene in nature and no glass material was used to prevent possible metal contamination. All the precautions were taken in accordance with the Clinical and Laboratory Standards Institute criteria [19] to eliminate metal contamination while collecting and storing the samples.

2.7. Instrumentation and elemental analysis

Elemental analysis was carried out using an inductively coupled plasma–atomic emission spectrometry (ICP–AES) model Jobin Yvon 38 sequential analyzer. The elements measured were Na, K, S, Ca, Mg, P, Cu, Fe, Zn, Mn and Al. All dilutions were made with ultra pure milliQ water (18-mega ohms resistance) in dust-free environment. The optimization of ICP–AES was carried out by line selection and detection limits for each element [20]. The validation of the analysis was tested by analyzing serum

Table 3
Trace element concentrations (μmol/mL) in serum samples of control and bipolar disorder

Elements	Reference values #	Control (*N*=25)	Bipolar type I (*N*=40)	%Change	Bipolar type II				Bipolar type V (N=30)	%Change
					Hypomania (*N*=25)	%Change	Depression (*N*=25)	%Change		
Na	134.78	133.53±7.5	153.57±17.9*	15%	156.11±6.5***	17%	150.83±9.5***	13%	142.17±10***	6.47%
K	4.09	3.27±0.8	4.28±1.44**	31%	4.09±0.4	25%	4.37±0.7**	33.6%	3.73 ±0.31	4.06%
S	NA	36.63±3.6	31.79±5.5*	−13%	34.40±2.8*	−6%	33.86±3.1*	−7.56%	31.37±4.51**	−14%
Ca	2.20	2.26±0.3	2.37±0.36	5%	2.56±0.1	13.2%	2.46±0.2	8.84%	2.42±0.15	7.07%
Mg	0.82	0.93±0.1	1.08±0.14	16%	1.04±0.1	11.87%	1.03±0.1	10.7%	1.03±0.09***	10.7%
P	3.55	3.16±0.4	3.77±0.67*	19.3%	3.45±0.4	9.2%	3.78±0.6	20%	3.91±0.61**	23.7%
Fe	0.021	0.02±0.01	0.0195±0.01***	−15.2%	0.020±0.01	−18.0%	0.018±0.01***	−22%	0.0153±0.01**	−34.7%
Cu	0.016	0.013±0.004	0.023±0.01*	76.9%	0.020±0.002	53.84%	0.020±0.01***	53.8%	0.020±0.01**	53.8%
Zn	0.013	0.0086±0.001	0.008±0.002***	−4.65%	0.0088±0.003	2.3%	0.0077±0.001**	−10.46	0.0071±0.002**	−17.4%
Al	0.00018	0.00050±0.001	0.0040±0.001*	578%	0.0010±0.0003**	100%	0.0012±0.001*	140%	0.0012±0.0004**	140%
Mn	NA	0.00031±0.0001	0.002±0.001*	486%	0.0009±0.00004*	190.3%	0.00029±0.0001	−6.45%	0.0003±0.0001	−3.22%
Total		179.82	196.91		201.71		196.38		184.68	

NA=Not available.
Statistically significant *$p<0.0001$; **$p<0.001$; ***$p<0.01$.
Reference values from handbook on metals in clinical and analytical chemistry. (Reference No: [22,23]).
Reference values for Na and K are from G.V. Iyengar "Elemental Analysis of Biological Systems. 1989. (Reference No: [24]).

matrix match multi-element synthetic standard and certified standard reference material (Bovine liver 1577a) obtained from the National Institute of Standards, as reported earlier [19,20]. The lines were selected for each element in such a way that interference from the other elements was at a minimum. The wavelength used and the detection limit of the elements are summarized in Table 2.

2.8. Statistical analysis of data

The concentrations of elements were expressed as μmol/ml with mean, SD, and *p*-value). The mole percentage (elemental concentration in mole percentage=elemental concentration (μmol/ml)×100/total elemental concentration (μmol/ml) of analyzed elements in each sample) was calculated for the analyzed elements and the relative distribution of these elements based on mole percentage was computed. Mole percentage calculations are essential for understanding the relative distribution of each element in relation to other elements in biological matrix and they also help to normalize the data of different samples in order to arrive at clear inter-element relationship [21]. Element-to-element ratios and correlations were calculated based on mole percentage in order to arrive at possible elemental inter-relationship (direct or inverse) both in control and bipolar patients' serum. The relative charge distribution in terms of single (Na, K), double (Mg, Cu, Zn, Ca) and triple (Al, Fe) charged ions distribution were calculated using the formula:

Sum of concentration of a particular element in μmol per ml×Z×Avogadro's number×10^{-6}; where Z is 1,2,3 for single, double, triple charged ions respectively.

All statistical calculations such as inter-relations, correlation coefficients and *t*-tests were carried out using statistical software packages.

3. Results

3.1. Elemental concentration

Macro and trace elemental concentration (μmol/ml) for the control group and bipolar groups (I, II, V) are tabulated in Table 3. The comparative account of trace elements between the control group and bipolar group patients' serum samples revealed the following trends: a) in the bipolar I patients, Na, K, P, Cu, Al and Mn were increased significantly ($p<0.001$), b) in bipolar II hypomania, Na, Mn, and Al were increased significantly ($p<0.01$), c) in bipolar II patients suffering from depression, Na, K, Cu, and Al were increased significantly ($p<0.001$) and d) in bipolar V depressive patients Na, Mg, P, Cu and Al were increased significantly ($p<0.001$). But elements namely S, Fe, and Zn concentrations were decreased significantly ($p<0.001$) in all 3 bipolar types. Further, bipolar I (manic) have higher concentrations of Cu, Al, and Mn than bipolar II hypomania. The total elemental

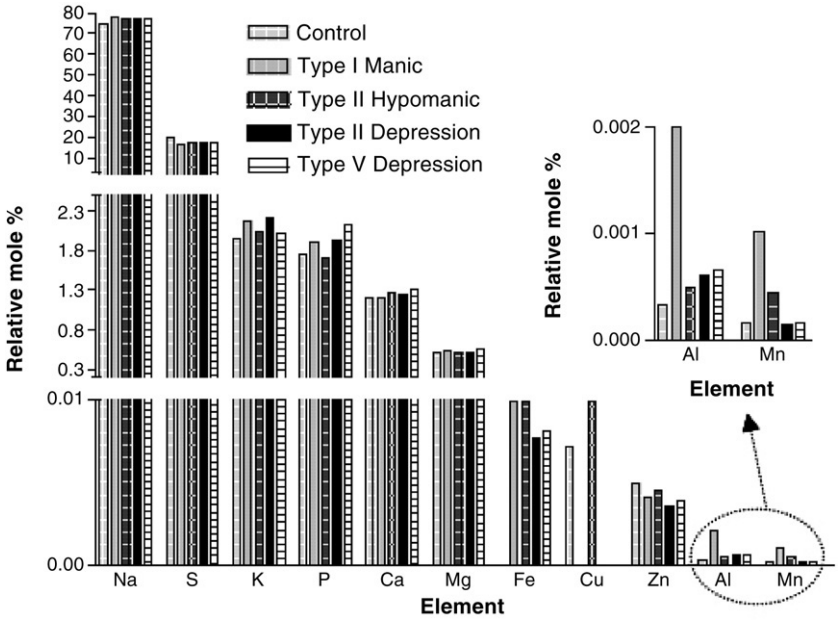

Fig. 1. Comparison of relative mole percentage of elements in control and Types I, II, and V bipolar disorders human serum samples.

concentration (μmol/ml) was higher ($p<0.04$) in the bipolar groups serum compared to the control group indicating possible imbalance in elemental homeostasis.

3.2. Relative mole percentage and mole percentage ratios

Relative mole percentage was calculated in order to normalize the concentration concentrations (μmol/ml) of each element in a sample of total elements analyzed. The relative distribution is represented in Fig. 1. The mole percentage data indicates that Fe, S and Zn concentrations were higher in control group serum compared to bipolar groups' serum. The concentrations of Na, K, Cu, P, Al and Mn were increased in bipolar I. The concentrations of Na, Al and Mn increased in bipolar II hypomanic and Na, K, Cu and Al are increased in bipolar II depression. The concentrations of Mg, P, Cu, Al concentrations were increased in bipolar V. Further, the concentrations of Cu, Al, and Mn were high in bipolar I (manic) compared to bipolar II and V. However, the concentrations of Fe were decreased in all 3 bipolar types.

The data are expressed in terms of element-to-element mole percentage in control, bipolar I, bipolar II, bipolar V serum samples in Table 4. These ratios help to understand the inter-relationship of elements in biological system. The element-to-element mole ratios namely, Na/Fe, K/Fe, Ca/Fe, Mg/Fe, Cu/Fe, and Cu/Zn were increased in serum samples of all 3 types of bipolar groups. Al/Fe, Al/Zn, ratios were increased in bipolar I (Manic patients) serum samples. The ratio of Al/Fe and Al/Zn was higher in bipolar I (mania) compared to others. The ratio of Al/Mn is lower in bipolar II (hypomania) compared to control. We observe a pattern in element-to-element displacement in bipolar disorders compared to control.

3.3. Inter elemental correlations: an insight into elemental homeostasis

Correlation coefficients were calculated using the concentration micromoles of elements of control group and bipolar groups. The results in Table 5 showed a distinct pattern of direct and inverse correlation. The direct relation (confidence concentration 95–99.7%) is observed between K vs (S, Ca, Mg, Fe, Cu), S vs (Ca, Mg, Fe), Ca Vs (Mg, Fe), and Mg vs Fe in serum samples of control group. The inverse correlation (confidence concentration 95%–99.7%) is also observed between Na vs (S, Cu, K, Ca, Mg, Fe), K vs (P, Zn), and P vs (Fe, Zn) in serum samples of control group. In bipolar I serum samples direct correlation (confidence concentration 95%–99.7%) is seen between K vs Zn, S vs Ca, Mg vs (Cu, Zn), P vs Fe and inverse correlation observed between Na vs (S, K, Ca) and K vs Al. In the serum samples of bipolar II hypomania direct correlation (95%–99.7% confidence concentration) is observed between Ca vs (Mg, Fe), P vs (Fe, Mn) and inverse correlation is seen between Na vs (S, Mn), K vs Al, S vs (Ca, Mg, Al), Ca vs Zn, Fe vs Zn, whereas the direct correlation (95%–99.7% confidence concentration) is observed between Ca vs (Mg, Fe), Fe vs Zn and an inverse correlation is seen between Na vs S, S vs Al in the serum samples of bipolar II depression.

Table 4
Element to element mole percentage ratio in serum samples of control, bipolar I,bipolar II and bipolar V mood disorders

Element ratio	Normal	Bipolar I (Mania)	Bipolar II (Hypomania)	Bipolar II (Depression)	Bipolar V depressives
Na/Fe	5674	7782	7310	8374	9172
K/Fe	139	213	200	242	241
Ca/Fe	96	120	120	136	156
Mg/Fe	39	55	49	57	66
Cu/Fe	0.55	1.17	0.82	1.13	1.28
Cu/Zn	1.52	2.83	2.02	2.62	2.78
Al/Fe	0.021	0.20	0.05	0.07	0.08
Al/Zn	0.057	0.49	0.12	0.15	0.16
Al/Mn	1.61	2.21	1.19	4.07	3.98

Table 5
Comparison of inter-elemental relations between controls, bipolar disorder typeI, II and V

Correlation between elements		Correlation co-efficient				
1	2	Control ($n=25$)	BD I ($n=40$)	BD II Hypomania ($n=25$)	BD II depression ($n=25$)	BD V ($n=30$)
Na	S	−0.97*	−0.95*	−0.97*	−0.96*	−0.97*
Na	Cu	−0.46**	NS	NS	NS	+0.57**
Na	Mn	NS	NS	−0.66*	NS	NS
Na	K	−0.70*	−0.32**	NS	NS	NS
Na	Ca	−0.63*	−0.37**	NS	NS	NS
Na	Zn	NS	−0.31**	NS	NS	NS
Na	Mg	−0.52**	NS	NS	NS	+0.36**
Na	Fe	−0.59**	NS	NS	NS	NS
K	S	+0.53**	NS	NS	NS	−0.39**
K	Ca	+0.58**	NS	NS	NS	+0.50**
K	Mg	+0.52**	NS	NS	NS	NS
K	P	−0.46**	NS	NS	NS	NS
K	Fe	+0.65*	NS	NS	NS	NS
K	Cu	+0.70*	NS	NS	NS	+0.43**
K	Zn	−0.46**	+0.42**	NS	NS	NS
K	Al	NS	−0.46**	−0.54**	NS	NS
S	Ca	+0.54**	+0.33**	NS	NS	NS
S	Mg	+0.40**	NS	NS	NS	−0.43**
S	Fe	+0.52**	NS	NS	NS	NS
S	Cu	NS	NS	NS	NS	−0.63*
S	Al	NS	NS	−0.50**	−0.45**	+0.33
S	Mn	NS	NS	+0.58**	NS	NS
Ca	Mg	+0.77*	NS	+0.40**	+0.42**	+0.54**
Ca	Fe	+0.74*	NS	+0.43**	+0.57**	+0.54**
Ca	Al	NS	NS	NS	NS	−0.48**
Ca	Zn	NS	NS	−0.66*	NS	NS
Mg	Fe	+0.43**	NS	NS	NS	+0.55**
Mg	Cu	NS	+0.49**	NS	NS	NS
Mg	Zn	NS	+0.33**	NS	NS	NS
P	Fe	−0.61*	+0.39**	+0.54**	NS	NS
P	Zn	NS	NS	NS	NS	−0.49**
P	Mn	NS	NS	+0.57**	NS	+0.61*
Cu	Al	NS	NS	NS	NS	−0.62*
Cu	Zn	NS	NS	NS	NS	−0.39**
Fe	Zn	NS	NS	−0.81*	+0.38**	NS

Note: Confidence levels: *>99.7%, **>95%, the expected correlation co-efficient for sample size of 25 are 0.613 and 0.396 respectively. Positive and negative signs indicate direct and inverse correlation respectively. NS=Not significant; BD=bipolar disorder.

In the serum samples of bipolar V direct correlation is observed (95%–99.7% confidence concentration) between Na vs Cu, K vs (Ca, Cu), Ca vs (Mg, Fe), Mg vs Fe,P vs Mn, and inverse relation is seen between Na vs S, K vs S, S vs (Mg, Cu), Ca vs Al, P vs Zn, Cu vs (Al, Zn). Overall few correlation coefficients differ from bipolar groups to control group. The direct correlation is seen between K vs Zn and P vs Fe in serum samples of bipolar I, but in control group serum samples K vs Zn and P vs Fe show an inverse relation. In bipolar II hypomania direct relation is observed between P vs Fe whereas in control serum it is in inverse relation. In bipolar V depressives Na vs Cu shows direct correlation while in control it is an inverse relation. Inverse relation is observed between K vs S and S vs Mg in serum samples of bipolar V but it shows direct relation in the control group.

4. Discussion

Macro and trace elements are significant either as causative or therapeutic factors in neurological and neuropsychiatric disorders [4]. The first breakthrough providing evidence of the therapeutic potential of trace elements in neuropsychiatry came with the use of Li in manic-depressive psychosis [12,13]. A similar therapeutic role has been proposed for Rb in an affective illness [25]. V and Al have been implicated as causative factors in bipolar mood disorder and dementia respectively [9,26]. This study showed that like Li, Mg also has therapeutic potential [27].

The data generated in this study on elemental concentration in control human subjects were compared with the reference elemental values in serum from Christenson et al. [22], Muniz et al. [23], and Iyengar [24]. However in the case of Al, it is different. The normal value for Al in serum sample appears to be an open question. Though it is agreed that Al concentration should be <0.005 μg/ml in control human serum, in many studies the values of around 0.010–0.020 μg/ml has been reported. The Al concentration in our present investigation for control human serum was 0.016 μg/ml which is well within the range of earlier reported values.

Our results show increased Cu concentration in 3 types of bipolar disorders (I, II and V). Naylor et al. [10] also showed a significant increase of Cu in the serum of manic patients. However, they observed no significant change in Na, S, Mg, Ca, Mn and Al in manic-depressive patients, while our results indicate a significant increase in Na, Mn and Al in manic and hypomanic patients and no change in Mn concentrations in case of bipolar II depression and bipolar V. Our results also demonstrate decreased Zn concentrations and increased Cu concentrations in 3 types of bipolar groups. Other studies have reported a similar trend in schizophrenic and major depressive patients [28–31]. Cu is an essential element for the activity of a number of physiologically important enzymes such as cytochrome C oxidase, dopamine-beta-hydroxylase, and Cu/Zn superoxide dismutase, which is critical in scavenging reactive oxygen species. Malfunctions related to any of these enzymes due to lack of Cu may contribute to pathophysiology of brain diseases [32]. In addition, higher concentrations of Cu also influence the generation of reactive oxygen species leading to Cu-mediated oxidative stress which will affect several intracellular alterations and contribute to cell death pathway [33].

Our results show higher concentrations of Al and lower concentrations of Fe in the serum of 3 bipolar types compared to control group. Interestingly, van Rensburg et al. [34] have shown Al to be significantly high and Fe to be low in serum of patients suffering from chronic fatigue syndrome which affects cognition and influences neurological abnormalities. The low concentrations of Fe in serum that we observed could indicate high concentrations of the same in the brain, as has been suggested earlier [20,21,35,36]. Our data also indicate higher concentrations of Mn in bipolar I manic and II hypomanic. Mn toxicity has been shown to be associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons [37]. We observed the DNA fragmentation in depression brain samples (unpublished data).

In the present investigation we observed that total concentrations of elements are relatively higher in the bipolar groups than the control group, indicating imbalance in the elemental homeostasis. We believe that increased Al concentration causes imbalance in trace elemental homeostasis pool resulting in an increase in the concentrations of Na, K, P, Cu and Mn and decrease in the concentrations of Fe, S, and Zn in the serum of the bipolar groups. The effect of an increase or decrease in a single element concentration is not restricted to this element alone, but the total element distribution pattern in the system will be affected. Thus interdependency in the concentration of certain elements to maintain homeostasis of trace elements pool which is apparent from our results seems to be crucial in a biological system.

Most of the studies conducted so far were primarily limited to individual trace elemental concentration only in neuropsychiatric disorders, and no attempt has thus far been made to understand the element-to-element inter-relationship in bipolar disorders. This

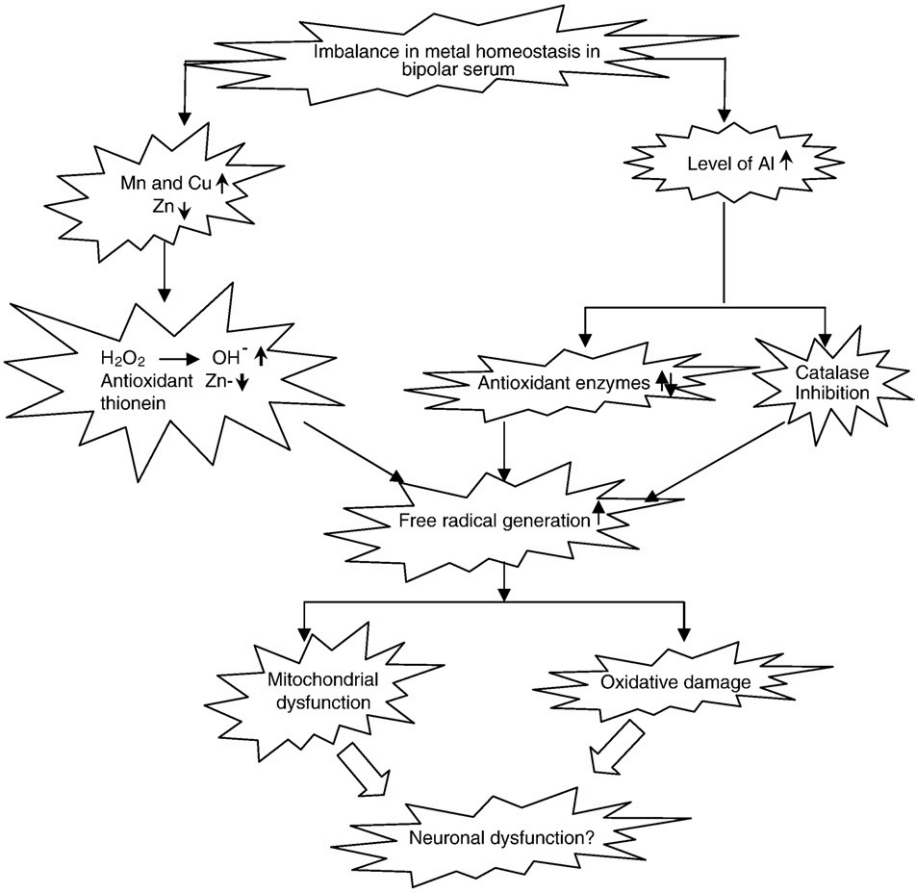

Fig. 2. Possible pathways for neuronal dysfunction associated with bipolar disorders due to imbalance in metal homeostasis. The figure advocates imbalance in trace elemental homeostasis leads to oxidative damage as prime risk factor for behavioral and neuronal dysfunction. Note: The upward and downward arrow marks indicating increasing and decreasing trend.

report is to our knowledge, the first of its kind to show the inter-elemental relations among elements in bipolar groups serum samples in comparison to a control group. From our analysis of the trace elemental concentration and element-to-element inter-relationship in 3 types of bipolar patients in comparison with the control group, a definite correlation pattern is observed. Further, our data expressing element-to-element mole percentage ratios in control, bipolar I, bipolar II, bipolar V serum samples provide an insight (see below) into the interdependency of elements in biological system [20].

Our results from the element-to-element ratio and correlation patterns indicate that there is a definite imbalance in the trace elemental homeostasis and element-to-element inter-relationship pattern in serum of bipolar groups compared to controls. It has been shown that element-to-element mole ratio provides clues to the possible inter-relationships of metals in the neurobiological system [20,21,34]. The high Cu/Zn ratios in bipolar disorders that we observed, is in agreement with the observation of Mazzetti et al. [38]. However, more significantly, Johnson [39] has demonstrated that the developing fetus of pregnant women who have low Zn and high Cu might experience major difficulties in the early development of the brain, which may later manifest into mental illness. The present study on assessment of serum elements in 3 types of bipolar disorder compared to control will help in linking the understanding of cellular processes such as oxidative stress, apoptosis and neuronal dysfunction.

Based on our new findings and earlier literature sources, we developed a hypothetical model to explain the possible relevance of macro and trace element homeostatic imbalance in serum of bipolar disorder to possible effects in brain (Fig. 2). We propose that increased Al concentration in serum of bipolar disorders is likely to alter the trace elemental homeostasis pool. We suggest that irrespective of elements being primary risk factors or consequences of disease mechanism, a change in an individual metal ion will upset the elemental homeostasis pool resulting in a significant imbalance in elemental concentrations and charge distribution pattern in the biological system.

The element-to-element mole ratio of Al/Fe and Al/Zn for instance is increased because of the high concentration of Al present and it alters other elemental concentrations. Increased Al may disturb metal homeostasis in serum by increasing the paramagnetic oxidant elements like Cu and Mn while decreasing Zn, an antioxidant metal required as cofactor for CuZn–SOD and Zn–thionein which are essential to prevent oxidative damage. Increased Al is found to increase superoxide dismutase (SOD) activity to protect the cell from oxidative stress. Kuloglu et al. [40] showed that SOD activity concentrations are higher in bipolar disorder serum with the presence of lipid peroxidation also being reported. Increased Al may therefore be one of the reasons for high SOD activity in bipolar disorder. Increased redox metals Cu and Mn concentrations in serum may catalyze the conversion of H_2O_2 to potent hydroxyl radical, which could lead to oxidative damage. Al not only promotes Fe-mediated oxidative stress by inhibiting catalase activity in the brain, but also causes increase in free radical generation leading to mitochondrial dysfunction, oxidative stress and neuron dysfunction [41]. It has also been implicated in the degeneration of cholinergic terminals in cortex and hippocampus [42].

This suggests that trace elemental imbalances in bipolar disorder serum may cause imbalances in trace elemental concentrations in the brain which may lead to oxidative damage to biomolecules. This may be the reason for alteration in the neurotransmitter receptors and the concentrations of secondary messenger in the bipolar disorder brain. Thus imbalance of the elemental homeostasis may play a role in etiology of bipolar disorder. Certain metals such as Zn, Al and Pb have been implicated in the aggregation of alpha-synuclein, a crucial protein in Parkinson's disease [43], while others such as Cu, Fe and Zn are involved in the precipitation and aggregation of beta-amyloid peptide in Alzheimer's disease [44,45]. Similar metal-ion-biomolecular related mechanisms could underlie the etiology of other neurological disorders like bipolar disorder. It is an agreed upon fact that a combination of environmental metal pollution and oxidative stress play an important role in triggering neurodegeneration [46,47]. Thus imbalanced elemental homeostasis causes an imbalance of oxidants/antioxidants which leads to neuronal dysfunction.

In this scenario, our data suggest that elemental homeostatic imbalance results in the imbalance of biochemical events and cause oxidative stress in bipolar disorder, which may later manifest into neurodegeneration. This hypothesis is supported by other studies: PET scanning [48] showed that depression might leads to neurodegeneration; it has also evidenced the presence of neuritic pathology in patients having bipolar mood disorder [49]. On the other hand, Damadzic et al. [50] observed no neuritic pathology in the entorhinal cortex, subiculum and hippocampus in middle-aged adults with schizophrenia, bipolar disorder or unipolar depression. We believe that neuritic pathology may be the final onset phase of neurodegeneration, with the initial phases being neuropsychiatric phenomena involving biochemical and brain functional alterations.

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References

[1] Strong MJ, Garruto RM. Experimental paradigms of motor neuron degeneration. In: Woodruff ML, Nonneman AJ, editors. Toxin-induced Models of Neurological Disorders. New York: Plenum Press; 1994. p. 39–88.
[2] Garruto RM, Flaten TP, Wakayama I. Natural and experimental models of environmentally induced neurodegeneration:Implication for Alzheimer's disease. In: Corain B, Wisneiwski H, Zatta P, editors. Alzheimer's Disease: Advances in Clinical and Basic Research. John Wiley and Sons Ltd; 1993. p. 257–66.
[3] Mertz W. Risk assessment of essential trace elements: new approaches to setting recommended dietary allowances and safety limits. Nutr Rev 1995;53:179–85.
[4] Linter CM. Neuropsychiatric aspects of trace elements. Br J Hosp Med 1985;34:361–5.
[5] Stanley PC, Wakwe VC. Toxic trace metals in the mentally ill patients. Niger Postgrad Med J 2002;9:199–204.
[6] Hansen CR, Malecha M, Mackenzie TB, Kroll J. Copper and zinc deficiencies in association with depression and neurological findings. Biol Psychiatry 1983;18:395–401.
[7] Ali SA, Peet M, Ward NI. Blood concentrations of vanadium, cesium and other elements in depressive patients. J Affect Disord 1985;9:187–91.
[8] Srinivasan DP, Marr S, Wareing RA, Birch NJ. Magnesium, zinc and copper in acute psychiatric patients. Magnes Bull 1982;4:45–8.
[9] Naylor GJ, Smith AH. Vanadium a possible etiological factor in manic-depressive illness. Psycho Med 1981;11:249–56.
[10] Naylor GJ, Smith AHW, Bryce-Smith D, Ward NI. Trace elements in manic depressive psychosis. J Affect Disord 1985;8:131–6.
[11] Campbell CA, Ward NI, Peet M. Increased bromide concentrations in serum and hair during lithium treatment. J Affect Disord 1986;11:161–4.
[12] Campbell CA, Peet M, Ward NI. Vanadium and other trace elements in patients taking lithium. Biol Psychiatry 1988;24:775–81.
[13] Harvey NS, Jarratt J, Ward NI. Trace elements and the electroencephalogram during long-term lithium treatment. Br J Psychiatry 1992;160:654–8.
[14] Christiansen C, Baastrup PC, Lindgreen P, Transbol I. Endocrine effects of Lithium:II 'primary' hyperparathyroidism. Acta Endocrinol 1978;88:528–34.
[15] American Psychiatric Association. Diagnostic and statistical manual of mental disorders. fourth ed. Washington, DC: American Psychiatric association; 1994.
[16] Young RC, Klerman GL. Mania in late life: focus on age at onset. Am J Psychiatry 1992;149:867–76.
[17] Fell GS, Burns RR. Zinc and other trace elements. Adv Parent Nutr 1981;16:241–61.
[18] Triplett WC. Clinical aspects of zinc, copper, manganese, chromium and selenium metabolism. Nutr Int 1985;1:60–7.
[19] National committee for Clinical Laboratory Standards Approved guidelines. Control of pre-analytical variation in trace element determination, vol. 17; 1997. p. 1–30.
[20] Rajan MT, Rao KSJ, Mamatha BM, et al. Quantification of trace elements in normal human brain by inductively coupled plasma atomic emission spectrometry. J Neurol Sci 1997;146:153–66.

[21] Rao KSJ, Rao RV, Shanmugavelu P, Menon RB. Trace elements in Alzheimer's disease brain: a new hypothesis. Alzheimer's Rep 1999;2:241–6.
[22] Christensen JM, Kristiansen J. Lead. In: Seiler HG, Siegel A, Sigel H, editors. Hand book on metals in clinical and analytical chemistry. New York: Marcel Dekker; 1994. p. 217–667.
[23] Muniz CS, Marchante-Gayon JM, Alonso JIG, Sanz-Medl A. Multi elemental trace analysis of human serum by double focusing ICP-MS, vol. 14; 1999. p. 193–8.
[24] Iyengar GV. Elemental analysis of biological system, vol. 1. Boca Raton: CRC press; 1989. p. 242.
[25] Fieve RR, Jamison KR. Rubidium: overview and clinical perspectives. Mod Probl Pharmacopsychiatry 1982;18:145–63.
[26] Alfrey AC, LeGendre GR, Kaehny WD. The dialysis encephalopathy syndrome possible aluminium intoxication. N Engl J Med 1976;294:184–8.
[27] Murck H. Magnesium and affective disorders. Nutr Neurosci 2002;5:375–89.
[28] Olatunbosun DA, Akindele BK, Adadevoh BK, Asuni T. Serum copper in schizophrenia in Nigerians. Br J Psychiatry 1975;127:119–21.
[29] Alias AG. Serum ceruloplasmin in schizophrenia:significant increase in acute cases especially in catatonia. Biol Psychiatry 1972;4:231–8.
[30] Pfeiffer CC, Ilier UA. Study of zinc deficiency and copper excess in the schizophrenias. Int Rev Neurobiol 1972;1:141–65.
[31] Diebel MA, Ehmann WD, Markesbery WR. Copper, iron and zinc imbalances severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. J Neurol Sci 1996;143:137–42.
[32] Strausak D, Mercer JF, Dieter HH, Stremmel W, Multhaup G. Copper in disorders with neurological symptoms: Alzheimer's, Menkes, and Wilson diseases. Brain Res Bull 2001;55:175–85.
[33] Carri MT, Ferri A, Cozzolino M, Calabrese L, Rotilio G. Neurodegeneration in amyotrophic lateral sclerosis: the role of oxidative stress and altered homeostasis of metals. Brain Res Bull 2003;61:365–74.
[34] van Rensburg SJ, Potocnik FC, Kiss T, et al. Serum concentrations of some metals and steroids in patients with chronic fatigue syndrome with reference to neurological and cognitive abnormalities. Brain Res Bull 2001;55:319–25.
[35] Rao KSJ, Shanmuga velu P, Shankar SK, et al. Trace elements in the cerebrospinal fluid in Alzheimer's disease. Alzheimer's Rep 1999;2:4–9.
[36] Thompson KJ, Shoham S, Connor JR. Iron and neurodegenerative disorders. Brain Res Bull 2001;55:155–64.
[37] Malecki EA. Manganese toxicity is associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons. Brain Res Bull 2001;55:225–8.
[38] Mazetti A, Pierdomenico SD, Costantini F, et al. Copper/Zinc ratio and systemic oxidant load: effect of aging and aging-related degenerative diseases. Free Radic Biol Med 1998;25:676–81.
[39] Johnson S. Micronutrient accumulation and depletion in schizophrenia, epilepsy, autism and Parkinson disease? Med Hypotheses 2001;56:641–5.
[40] Kuloglu M, Ustundag B, Atmaca M, Canatan H, Tezcan AE, Cinkilinc N. Lipid peroxidation and antioxidant enzyme concentrations in patients with schizophrenia and bipolar disorder. Cell Biochem Funct 2002;20:171–5.
[41] Savory J, Rao KSJ, Huang Y, Letada PR, Herman MM. Age-related hippocampal changes in Bcl-2:Bax ratio, oxidative stress, redox-active iron and apoptosis associated with aluminum-induced neurodegeneration: increased susceptibility with aging. Neurotoxicology 1999;20:805–17.
[42] Platt B, Fiddler G, Riedel G, Henderson Z. Aluminium toxicity in the rat brain: histochemical and immunocytochemical evidence. Brain Res Bull 2001;55:257–67.
[43] Yamin G, Glaser CB, Uversky VN, Fink AL. Certain metals trigger fibrillation of methionine-oxidized alpha-synuclein. J Biol Chem 2003;278:27630–5.
[44] Bush AI. The metallobiology of Alzheimer's disease. Trends Neurosci 2003;26:207–14.
[45] Kawahara M, Kato M, Kuroda Y. Effects of aluminium on the neurotoxicity of primary cultured neurons and on the aggregation of beta-amyloid protein. Brain Res Bull 2001;55:211–7.
[46] Rao AV, Balachandran B. Role of oxidative stress and antioxidants in neurodegenerative diseases. Nutr Neurosci 2002;5:291–309.
[47] Campbell A, Smith MA, Sayre LM, Bondy SC, Perry G. Mechanisms by which metals promote events connected to neurodegenerative diseases. Brain Res Bull 2001;55:125–32.
[48] Bier JC, Fery P, Elincx S, Claes T, Ventura M, Goldman S. Depression may mimic the clinical and metabolic patterns of degenerative disease. Alzheimer's Rep 2002;5:41–4.
[49] Buhl L, Bojsen-Moler M. Frequency of Alzheimer's disease in a postmortem study of psychiatric patients. Dan Med Bull 1988;35:288–90.
[50] Damadzic R, Shuangshti S, Giblen G, Herman MM. Neuritic pathology is lacking in the entorhinal cortex, subiculum and hippocampus in middle-aged adults with schizophrenia, bipolar disorder or unipolar depression. Acta Neuropathol 2002;103:488–94.