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ORIGINAL ARTICLE

# Total and ionized calcium and magnesium are significantly lowered in drug-naïve depressed patients: effects of antidepressants and associations with immune activation

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## Abstract

Major depressive disorder (MDD) is associated with alterations in calcium (Ca) and magnesium (Mg), as well as circulating pro- and anti-inflammatory cytokines. Anti-inflammatory drugs are commonly used as adjuvant treatments for MDD. However, no studies examined the effects of a combinatorial treatment with sertraline and ketoprofen, an anti-inflammatory drug, on Ca and Mg levels in MDD. The present study examined a) differences in both cations between drug-naïve MDD patients and controls, and b) the effects of sertraline and ketoprofen on Ca and Mg (both total and ionized). In the same patients, we also examined the associations between both cations and IL-1β, IL-4, IL-6, IL-18, IFN-γ, TGF-β1, zinc, and indoleamine 2,3-dioxygenase (IDO). Clinical improvement was assessed using the Beck Depression Inventory-II (BDI-II) at baseline and after follow up for 2 months. Serum Ca and Mg (total and ionized) were significantly lower in MDD patients as compared with controls, while treatment significantly increased calcium but decreased magnesium levels. There were significant and inverse correlations between the BDI-II scores from baseline to endpoint and Ca (both total and ionized), but not Mg, levels. The effects of calcium on the BDI-II score remained significant after considering the effects of zinc, IDO and an immune activation z unit-weighted composite score based on the sum of all cytokines. There was a significant and inverse association between this immune activation index and calcium levels from baseline to endpoint. In conclusion, lowered levels of both cations play a role in the pathophysiology of major depression. Antidepressant-induced increases in Ca are associated with clinical efficacy and attenuation of the immune response. The suppressant effect of antidepressants on Mg levels is probably a side effect of those drugs. New antidepressant treatments should be developed that increase the levels both Ca and Mg.

**Keywords** Depression · Cytokines · Inflammation · Indoleamine 2,3-dioxygenase · Neuro-immune

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## Introduction

Major depressive disorder (MDD) is a common psychiatric disease with a prevalence varying between 0.4 and 15.7% across countries (Rai et al. 2013). The etiology and pathophysiology of MDD is associated with many different factors including psychological stress (Dold et al. 2019), immune activation (Maes and Carvalho 2018), changes in endogenous opioids (Al-Fadhel et al. 2019), genetic factors (Czarny et al. 2015), oxidative and nitrosative stress (Maes et al. 2011) and changes in minerals, elements and anti-oxidants (Maes et al. 1999). Among the elements examined in MDD, calcium (Ca) and magnesium (Mg) showed inconsistent results (Joffe et al. 1996; Jamilian et al. 2013; Styczeń et al. 2015; Deb et al. 2016; Islam et al. 2018).

Lowered levels of Mg are associated with the onset of depression, anxiety, and behavioral and personality changes (Wacker and Parisi 1968). Mg is an essential mineral that plays a key role in many bodily functions and regulates over 300 biochemical reactions, while 3571 human proteins potentially bind to Mg (Piovesan et al. 2012). Mg deficiency increases the risk of depression and is often accompanied by a variety of depressive symptoms (Serefko et al. 2013; Boyle et al. 2017). An association between a reduced content of Mg in the brain and depression was reported by Sowa-Kucma et al. (2013). Mg deficiency affects brain chemistry, membrane fluidity and inflammation (De Baaij et al. 2015; Phelan et al. 2018) all of which are associated with depression. Furthermore, Mg is also involved in the glutamatergic system, regulating learning, memory, neuroplasticity and perhaps antidepressant activity (Marsden 2011). Mg supplements may be used as an adjuvant in the treatment of MDD (Schwalfenberg and Genuis 2017).

Ca exists in blood in three forms: bound to proteins mainly albumin, complexed with organic and inorganic anions, or in ionized form, which represents about half of the total calcium (Baird 2011). In many disorders, ionized Ca is the biologically active form (Sava et al. 2005; Al-Hakeim and Alhillawi 2018). Depression is associated with conditions that are characterized by changes in Ca homeostasis including lowered vitamin D (Milaneschi et al. 2014) and hypoparathyroidism (Rosa et al. 2014). Mandel (1960) noticed that abnormal levels of Ca, both high and low, can lead to neuropsychiatric disorders including depression. Other results suggest that lower Ca levels may be inversely associated with neuropsychological performance in MDD (Grützner et al. 2018) while Sharma et al. (2017) reported a positive association between serum Ca and daily-life activities and neuropsychological functions in depression.

There is evidence that proinflammatory and anti-inflammatory cytokines are elevated in sera of patients with MDD (Schiepers et al. 2005; Al-Hakeim et al. 2015, Al-Hakeim et al. 2018b; Maes and Carvalho 2018). These results show that MDD is not only associated with elevated production of pro-inflammatory mediators, but also by mediators which have an immune-regulatory or anti-inflammatory effect and which may down-regulate the primary immune-inflammatory response. This new concept was named the "compensatory immune regulatory system" (CIRS) (Maes and Carvalho 2018). However, the associations between changed cation levels and activation of IRS/CIRS pathways has not been studied yet.

The role of immune-inflammatory pathways in MDD has encouraged researchers to examine the clinical efficacy of anti-inflammatory compounds in combination with antidepressants in the treatment of MDD (Kohler et al. 2019; Al-Hakeim et al. 2018a). A meta-analysis, which examined anti-inflammatory drugs in MDD, showed that these treatments may have some clinical efficacy as compared with placebo (Köhler et al. 2014, 2018). Nevertheless, there are no data whether treatments with sertraline with or without ketoprofen significantly affect Ca and Mg in association with clinical recovery. Ketoprofen is a non-specific cyclo-oxygenase (COX) inhibitor, which inhibits COX-1 and -2 thereby decreasing the production of proinflammatory prostaglandin precursors (Cryer and Feldman 1998). Previously, we have shown that the clinical improvement in MDD during treatment with sertraline (and ketoprofen or placebo) was significantly associated with increments in zinc levels and reductions in indoleamine 2,3-dioxygenase (IDO) and immune activation (Al-Hakeim et al. 2018c; Twayej et al. 2019). However, no research examined whether changes in Ca and Mg levels during antidepressant treatment are associated with clinical improvement beyond and above the effects of zinc, immune induces and IDO.

Hence, the present study was carried out to examine a) differences in both cations (total and ionized forms) between drug-naïve MDD patients and healthy controls; b) the effects of combinatorial treatment with sertraline and ketoprofen on Ca and Mg (both total and ionized) levels and c) whether changes in Ca and Mg during treatment are associated with clinical improvement beyond and above the effects of zinc, immune activation, and IDO.

## Subjects and methods

### Participants

In this study, we included one hundred and forty MDD patients and 40 healthy controls. The participants were recruited (November 2016 until August 2017) at the Psychiatry Unit, Al-Hakeem General Hospital and a private psychiatric clinic, Najaf Governorate, Iraq. Senior psychiatrists made the diagnosis of MDD according to ICD-10 (10th revision of the International Statistical Classification of Diseases and Related Health Problems)

criteria. All participants were assessed using a semi-structured interview, which comprised medical and psychiatric history. Patients and controls were excluded for any systemic disease including (auto)immune disorders, diabetes mellitus, inflammatory bowel disorder, and liver and kidney diseases. They were also excluded for neuro-inflammatory disorders including multiple sclerosis, stroke and Parkinson's disorder. We also excluded patients with another axis I disorder including schizophrenia, psycho-organic syndromes, and substance use. We also excluded subjects who used immune-modulatory drugs including glucocorticoids and cyclo-oxygenase inhibitors. Only occasional use of paracetamol was allowed. Controls were excluded for a current or lifetime diagnosis of psychiatric disorders. Furthermore, C-reactive protein (CRP) was, in all subjects, lower than 6 mg/L which excludes overt inflammation.

The present study consists of two parts: a) a case-control study including 140 MDD patients and 40 controls; and b) a prospective study that examines 44 of the 140 depressed patients in a prospective study during 2 months with blood samplings both before (baseline) and during treatment with sertraline with and without ketoprofen. The latter was part of a randomized trial recorded in the NIH US Library of Medicine, ClinicalTrials.gov Identifier: NCT03514810. No biomarker data or BDI-II scores were missing in these 44 patients. Sixteen of the patients received sertraline (start dose was 50 mg orally / day) and twenty-eight were treated with ketoprofen (100 mg orally once daily). Colored capsules (Caps and Chemicals, India) were filled with ketoprofen (Menarini Int., Italy) and/or sertraline (Actavis, Italy) and patients took the full dose in one capsule after breakfast. The protocol was approved by the IRB of the University of Kufa (#221, June 2016). The patients or their close first-degree relatives provided informed consent in accordance with the procedures outlined by the current IRB.

### Measurements

We used the Beck-Depression Inventory (BDI-II) score to assess the severity of depression (Hautzinger et al. 2006). The BDI-II was completed before treatment (baseline condition) in all patients and after treatment in the 44 MDD patients who were entered in the prospective part of the study. Blood samples were aspirated without a tourniquet and centrifuged for 5 min (3000 rpm) and, subsequently, sera were kept at −80 °C until thawed for assay. Serum Calcium was measured using a kit supplied by Biolabo® Co, France. Ca with Arsenazo III [1,8-Dihydroxy-3,6-disulpho-2,7-naphthalene-bis (azo)-dibenzenearsonic acid], at slightly acidic pH (6.8), yields a blue colored complex and its intensity is proportional to Ca concentrations. Serum albumin was measured in order to correct or adjust the Ca and Mg levels for changes in albumin. Serum albumin was assayed by bromocresol green at 630 nm. Corrected calcium was calculated using the formula: Corrected Ca (mg/dl) = T.Ca + 0.8[4-Albumin] (Ohbal et al. 2014). Ionized calcium was calculated using the following formula: $I.Ca^{2+} = 0.813 \times T.Ca^{0.5} – 0.006 \times Albumin^{0.75} + 0.079$ (Mateu-de Antonio 2016), which give the best approximate result. Serum Mg was estimated using the calmagite method supplied by Biolabo® Co, France. Mg forms a purple colored complex when treated with calmagite in alkaline solution measured at 520 nm. In the presence of EGTA, the reaction is specific and the intensity of the purple color is proportional to the Mg concentration. Serum ionized Mg levels were calculated according to the following formula: I.Mg (in mM) = (0.66 × (T.Mg in mM)) + 0.039 (Koch et al. 2002). Serum indoleamine-2,3-dioxygenase activity (IDO) was measured using an ELISA kit supplied by MyBioSource® Inc., USA. The assays of IL-1β, IL-6, IL-18, IL-4, IFN-γ, TGF-β1 were performed using ELISA kits (MyBioSource® Inc., and BioAssay Systems®, USA) and those methods were described in detail somewhere else (Twayej et al. 2019).

### Statistical analysis

Analysis of variance (ANOVA) was employed to assess differences in scale variables between diagnostic groups and analysis of contingency tables ($\chi^2$ test) to check associations between categorical variables. We used Pearson's product moment correlation coefficients to examine associations between scale variables. Multivariate general linear model (GLM) analysis was used to assess the effects of diagnosis (independent variable) on biomarkers (dependent variables) while adjusting for extraneous variables (age, sex, BMI). Repeated measurement data were analyzed using generalized estimating equation (GEE) tests examining the effects of treatment on Ca and Mg levels with effects of time (pre- versus post-treatment), treatment (sertraline versus sertraline + ketoprofen) and time X treatment. Tests were two-tailed and a $p$ value of 0.05 was used for statistical significance. All statistical analyses were performed using IBM SPSS windows version 25. We used z unit-weighted composite scores (Maes and Carvalho 2018) to obtain different immune indices. An immune activation index was computed as z value of IL-1 (zIL-1) + zIL-6 + zIL-18 + zIFN-γ + zIL-4 + zTGF-β1 (M1 + Th1 + Th2 + Treg reflecting the combined activity of macrophagic M1, Thelper-1, Thelper-2 and Tregulatory functions). We also computed zIL-1 + zIL-6 + zIL-18 + zIFN-γ, which reflects activation of M1 and Th1 phenotypes (M1 + Th1). Another index used is the zIL-4 + zTGF-β1 composite score reflecting regulatory mechanisms (Th2 + Treg). We computed the ratio of z(zIL-1 + zIL-6 + zIL-18 + zIFN-γ) - z(zIL4 + zTGF-β1), which reflects the pro-inflammatory / immune regulatory ratio.

## Results

### Socio-demographic data

Table 1 shows the socio-demographic and clinical data as well as Ca and Mg measurements in MDD patients and controls. We found no differences in sex, age, BMI or employment status between both categories. In the MDD group, there were significantly more single subjects and smokers than in the control group. Serum T.Mg and I.Mg were significantly reduced in MDD as compared with controls. T.Ca, corrected-Ca and I.Ca were all significantly lower in MDD than in controls. There were no significant differences in the T.Mg / T.Ca ratio between the two study groups, while the I.Mg / I.Ca ratio was significantly lower in MDD patients than in controls. There were no significant differences in serum albumin between both study groups.

We found no significant association between T.Mg and T.Ca ($r = 0.105$, $p = 0.159$, $n = 180$), while there was a weak significant inverse correlation between I.Mg and I.Ca ($r = -0.223$, $p = 0.003$, $n = 180$). There were no significant associations between the BDI-II score and any of the Mg and Ca data. There was a significant positive correlation between albumin and T.Mg ($r = 0.504$, $p < 0.001$, $n = 180$) and a weak but significant inverse correlation between albumin and T.Ca ($r = -0.157$, $p = 0.035$, $n = 180$). There were significant associations between albumin and I.Ca ($r = -0.707$, $p < 0.001$, $n = 180$) and corrected Ca ($r = -0.640$, $p < 0.001$, $n = 180$). Thus, the "corrected" values are strongly and inversely associated with albumin values, indicating that the "correction" equations used are not very adequate. Therefore, we will present the ionized and corrected Ca data in baseline conditions and compute the residualized Mg and Ca values obtained by regression of the cations on albumin. Also, because I.Mg is statistically redundant we will show the I.Mg values only in baseline conditions but will compute the residualized Mg values after regression on albumin.

### Differences in the Ca and Mg between MDD and controls

Table 2 displays the results of a multivariate GLM analysis with T.Mg, T.Ca, corrected Ca, I.Ca, and the T.Ca / T.Mg and I.Ca / I.Mg ratios as dependent variables, while the diagnosis was the primary explanatory variable. The results were controlled for possible effects of sex, age, and BMI. There was a highly significant effect of diagnosis with an effect size of 0.233 and also a moderate effect of age with an effect size of 0.106. Sex and BMI had no significant effects on the biomarkers. Tests for between-subjects effects showed that diagnosis was significantly associated with T.Mg, T.Ca, corrected Ca, I.Ca and I.Ca / I.Ca ratio. Tests for between-subject effects showed that there was a very weak albeit significant negative association between age and I.Ca / I.Mg ratio ($F = 4.50$, $df = 1/175$, $p = 0.035$). Smoking ($F = 2.00$, $df = 7/168$, $p = 0.057$) did not have a significant effect on these 6 biomarkers, while also tests for between-subjects effects showed that there were no significant effects of smoking on any of the 6 biomarkers (even without p-correction). Table 3 shows the model-generated estimated marginal

**Table 1** Socio-demographic, clinical and biomarker data in healthy controls (HC) and major depressed (MDD) patients in baseline conditions

| Variables | HC $n = 40$ | MDD $n = 140$ | F/X/ψ | df | p |
|---|---|---|---|---|---|
| Age (years) | 40.3(7.9) | 39.0(10.6) | 0.53 | 1/178 | 0.469 |
| BMI (kg/m$^2$) | 27.72(4.16) | 27.55(16.81) | 0 | 1/178 | 0.949 |
| Sex (F/M) | 18/22 | 62/78 | 0 | 1 | 0.936 |
| Smoker (N/Y) | 40/0 | 82/58 | 24.45 | 1 | 0.001 |
| Employment (Y/N) | 22/18 | 59/81 | 2.08 | 1 | 0.149 |
| Married / Single | 18/22 | 89/51 | 4.45 | 1 | 0.035 |
| T.Mg (mM) | 0.805(0.147) | 0.714(0.104) | 19.51 | 1/178 | <0.001 |
| I.Mg (mM) | 0.571(0.097) | 0.511(0.688) | 19.51 | 1/178 | 0.001 |
| T.Ca (mM) | 2.400(0.107) | 2.249(0.171) | 28.04 | 1/178 | 0.001 |
| Corrected Ca (mM) | 2.394(0.138) | 2.255(0.222) | 13.98 | 1/178 | 0.001 |
| I.Ca (mM) | 1.240(0.040) | 1.204(0.067) | 10.76 | 1/178 | 0.001 |
| T.Mg / T.Ca | 0.336(0.060) | 0.320(0.053) | 2.77 | 1/178 | 0.098 |
| I.Mg / I.Ca | 0.461(0.082) | 0.427(0.069) | 6.98 | 1/178 | 0.009 |
| Albumin (g/l) | 40.31(3.41) | 39.69(5.72) | 0.42 | 1/178 | 0.520 |

All data are shown as mean (SD)

T. Mg: Total magnesium; T. Ca: Total calcium; I. Mg: Ionized magnesium; I. Ca: Ionized calcium

Corrected Ca: Corrected calcium

**Table 2** Associations between biomarkers and diagnosis

| Tests | Dependent variables | Independent variables | F | df | P | Partial $\eta^2$ |
|---|---|---|---|---|---|---|
| Multivariate | T.Mg, T.Ca, Corrected Ca, I.Ca, T.Ca / T.Mg, I.Ca / I.Mg | Diagnosis | 8.59 | 2/166 | 0.001 | 0.233 |
| | | Sex | 0.89 | 2/166 | 0.503 | 0.030 |
| | | Age | 3.35 | 2/166 | 0.004 | 0.106 |
| | | BMI | 0.54 | 2/166 | 0.775 | 0.019 |
| Between-subject effects | T.Mg | Diagnosis | 18.75 | 1/175 | <0.001 | 0.097 |
| | T.Ca | Diagnosis | 27.61 | 1/175 | 0.001 | 0.136 |
| | Corrected Ca | Diagnosis | 14.67 | 1/175 | 0.001 | 0.077 |
| | I.Ca | Diagnosis | 11.54 | 1/175 | 0.001 | 0.062 |
| | T.Ca / T.Mg | Diagnosis | 2.59 | 1/175 | 0.11 | 0.015 |
| | I.Ca / I.Mg | Diagnosis | 6.54 | 1/175 | 0.011 | 0.036 |
| Univariate | M1 + Th1 + Th2 + Treg | Diagnosis | 122.14 | 1/174 | <0.001 | 0.511 |
| | M1 + Th1 | Diagnosis | 191.19 | 1/174 | 0.001 | 0.524 |
| | Th2 + Treg | Diagnosis | 45.77 | 1/175 | <0.001 | 0.207 |
| | M1 + Th1/Th2 + Treg | Diagnosis | 13.34 | 1/174 | 0.001 | 0.071 |
| | IDO | Diagnosis | 11.7 | 1/175 | 0.001 | 0.059 |
| | Zinc | Diagnosis | 14.51 | 1/168 | 0.001 | 0.080 |
| | Albumin | Diagnosis | 0.23 | 1/175 | 0.63 | 0.001 |
| Multivariate | Ca, Mg | Diagnosis | 23.41 | 2/173 | <0.001 | 0.213 |
| | | Albumin | 36.45 | 2/173 | 0.001 | 0.296 |
| Between-subject effects | Mg | Diagnosis | 22.45 | 1/174 | <0.001 | 0.114 |
| | | Albumin | 61.91 | 1/174 | 0.001 | 0.262 |
| | Ca | Diagnosis | 29.44 | 1/174 | 0.001 | 0.145 |
| | | Albumin | 6.49 | 1/174 | 0.012 | 0.036 |

All results of multivariate general linear model analysis with the biomarkers as dependent variables and diagnosis as explanatory variable while adjusting for extraneous variables including sex, age and body mass index (BMI)

T. Mg: Total magnesium; T. Ca: Total calcium; I. Mg: Ionized magnesium; I. Ca: Ionized calcium; Corrected Ca: Corrected calcium

M1 + Th1 + Th2 + Treg: immune activation index; computed as z value of interleukin (IL)-1 (zIL-1) + zIL-6 + zIL-18 + z interferon (IFN)-γ + zIL-4 + z transforming growth factor (TGF)-β1

M1 + Th1: index of M1 macrophage and Thelper (Th)-1 activities; computed as zIL-1 + zIL-6 + zIL-18 + zIFN-γ

Th2 + Treg: index of regulatory Th2 and Tregulatory (Treg) functions; computed as zIL-4 + zTGF-β1

M1 + Th1/Th2 + Treg: index of immune activation versus regulation; computed as z(zIL-1 + zIL-6 + zIL-18 + zIFN-γ) − z(zIL-4 + zTGF-β1)

mean values of the 6 biomarkers. Here we present the z values of the biomarker values, which were additionally adjusted for age, sex, and BMI. We found that T.Mg, T.Ca, I.Ca and corrected Ca and the I.Ca / I.Mg ratio were significantly lower in MDD than in controls and that the distance between both groups for T.Ca was 0.886 standard deviations.

Table 2 shows also the results of univariate GLM analyses with the other immune biomarkers as dependent variables and diagnosis as an explanatory variable while adjusting for possible effects of sex, age, and BMI. Table 3 shows the model-generated estimated marginal means in z scores. The univariate analyses showed significantly higher composite scores of immune activation, M1 + Th1, Th2 + Treg, M1 + Th1/Th2 + Treg ratio and IDO in MDD versus controls, while zinc was significantly lower in MDD than controls. There was a particularly strong impact of the diagnosis on the M1 + Th1 + Th2 + Treg and M1 + Th1 indices with effect sizes of 0.511 and 0.524, respectively. There were no significant differences in albumin between both groups.

Table 2 shows also the T.Ca and T.Mg values corrected for serum albumin levels, thus reflecting the unbound (or ionized) forms of Ca and Mg. Toward this end, we have performed a multivariate GLM analysis with T.Ca and T.Mg as dependent variables and diagnosis and albumin as primary explanatory variables, while adjusting for age, sex and BMI. This GLM analysis showed that both T.Ca and T.Mg were significantly predicted by diagnosis and albumin. Table 3 shows the residualized T.Ca and T.Mg values (reflecting the unbound levels independent of albumin). Both the residualized T.Ca and T.Mg values were significantly lower in MDD than in

**Table 3** Differences in biomarkers between patients with major depression (MDD) and controls

| Variables | Controls | MDD |
|---|---|---|
| T. Mg | 0.577(0.152) | −0.167(0.149) |
| T. Ca | 0.694(0.149) | −0.192(0.08) |
| Corrected Ca | 0.521(0.153) | −0.142(0.082) |
| I. Ca | 0.466(0.154) | −0.125(0.082) |
| T. Ca / T. Mg | 0.220(0.159) | −0.069(0.085) |
| I. Ca / I. Mg | 0.349(0.156) | −0.104(0.084) |
| M1 + Th1 + Th2 + Treg | −1.345(0.114) | 0.386(0.062) |
| M1 + Th1 | −1.357(0.113) | 0.389(0.061) |
| Th2 + Treg | −0.831(0.146) | 0.250(0.079) |
| M1 + Th1/Th2 + Treg | −0.492(0.158) | 0.140(0.086) |
| IDO | −0.455(0.152) | 0.119(0.082) |
| Zinc | 0.538(0.156) | −0.147(0.085) |
| Albumin | 0.063(0.155) | −0.022(0.083) |
| Residualized Mg * | 0.546(0.131) | −0.156(0.07) |
| Residualized Ca * | 0.705(0.147) | −0.196(0.079) |

This table shows the model-generated estimated marginal means values (obtained by the multivariate general linear model analyses shown in Table 2) in controls and MDD patients. All data are shown as z scores (SE)

*Residualized values obtained after the regression on albumin levels

T. Mg: Total magnesium; T. Ca: Total calcium; I. Mg: Ionized magnesium; I. Ca: Ionized calcium; Corrected Ca: Corrected calcium

IDO: indeoleamine-2,3-dioxygenase

M1 + Th1 + Th2 + Treg: immune activation index; computed as z value of interleukin (IL)-1 (zIL-1) + zIL-6 + zIL-18 + z interferon (IFN)-γ + zIL-4 + z transforming growth factor (TGF)-β1

M1 + Th1: index of M1 macrophage and Thelper (Th)-1 activities; computed as zIL-1 + zIL-6 + zIL-18 + zIFN-γ

Th2 + Treg: index of regulatory Th2 and Tregulatory (Treg) functions; computed as zIL-4 + zTGF-β1

M1 + Th1/Th2 + Treg: index of immune activation versus regulation; computed as z(zIL-1 + zIL-6 + zIL-18 + zIFN-γ) – z(zIL-4 + zTGF-β1)

controls and the difference between MDD and controls was 0.901 and 0.702 standard deviations, respectively.

### Effects of sertraline and ketoprofen on Ca and Mg

Table 4 shows the demographic and clinical measurements at baseline among patients treated with ketoprofen and/or sertraline. We found no significant differences in BDI-II, sex, age, BMI, and smoking behavior between both groups. There were no significant differences in T.Mg and the residualized Mg (adjusted for albumin) and T.Ca and residualized Ca (adjusted for albumin) values between both study groups. There were no differences in albumin levels between both study groups.

Table 5 displays the results of GEE analyses, repeated measures, with effects of time (pre- versus post-treatment), treatment (sertraline versus sertraline + ketoprofen) and the time X treatment interaction on BDI-II and the biomarkers, while adjusting for sex, age, smoking, and BMI. We published previously (Al-Hakeim et al. 2018a) that sertraline + ketoprofen (pre-treatment and post-treatment means (SE): 48.82 ± 1.98 and 13.43 ± 1.26, respectively) reduced the BDI-II more than sertraline + placebo (49.94 ± 2.00 and 19.69 ± 1.70, respectively). There was a weak albeit significant effect of time (treatment) on T.Mg and the residualized Mg values (both lowered) and a significant positive effect of time on T.Ca and the residualized Ca values, while no significant time X treatment interactions could be established for both Mg and Ca values.

Using GEE analysis, repeated measurements, we have also examined the associations between BDI-II values from pre- to post-treatment condition (as dependent variables) and the Ca and Mg biomarkers (explanatory variables). Table 6, analysis #1 shows that there was no significant association between BDI-II values and T.Mg values (as well as residualized Mg, not shown). We found a significant negative correlation between T.Ca (GEE #2), but not residualized Ca (GEE #3), and the BDI-II score from baseline to post-treatment. We have also examined whether the pre- and post-treatment Ca values contribute to the prediction of the BDI-score and, therefore, entered the residualized Ca values together with M1 + Th1 + Th2 + Treg index, IDO and zinc as explanatory variables. GEE analysis #4 shows that these 4 different biomarkers had a significant effect on the BDI-II score. Finally, GEE analysis #5 shows that the residualized Ca data are significantly and inversely associated with the M1 + Th1 + Th2 + Treg index.

## Discussion

### Differences in Mg and Ca between MDD patients and controls

The first major finding of this study is that MDD patients show significantly lower levels of serum total and unbound Mg than controls. These results are in agreement with those of previous studies that serum Mg is reduced in MDD patients (Zieba et al. 2000; Cheungpasitporn et al. 2015) and that the incidence of hypomagnesemia is elevated in MDD patients as compared with controls (Levine et al. 1999). Alcohol use, chronic stress and certain diets (e.g. diets rich in fats and carbohydrates) may cause Mg deficiency in association with depressive symptoms (Tarleton et al. 2017), suggesting that reduced Mg levels participate in the pathophysiology of MDD (Rajizadeh et al. 2016; Islam et al. 2018). Importantly, lowered Mg levels in MDD may be caused by decreased appetite and Mg-impoverished diets (Jacka et al. 2009), while Mg-rich diets may reduce depressive symptoms (Derom et al. 2013). Lowered Mg levels may have some important consequences. Firstly, Mg deficiency can result in hypocalcemia through reductions in parathyroid hormone levels as a consequence

**Table 4** Sociodemographic, clinical, and biomarker data in the baseline (pre-treatment) condition in depressed patients who were allocated to the sertraline+placebo or sertraline+ketoprofen study groups

| Variable | Sertraline alone | Sertraline + Ketoprofen | $F/\chi^2$ | df | p |
|---|---|---|---|---|---|
| BDI-II | 49.9(8.3) | 48.8(10.7) | 0.13 | 1/42 | 0.720 |
| Age (years) | 38.5(11.8) | 39.4(10.1) | 0.07 | 1/42 | 0.801 |
| BMI ($kg/m^2$) | 25.61(5.37) | 26.89(4.35) | 0.75 | 1/42 | 0.392 |
| Sex (F/M) | 6/10 | 10/18 | 0.01 | 1 | 0.906 |
| Smoker (N/Y) | 10/21 | 6/7 | 0.76 | 1 | 0.382 |
| T. Mg (mM) | 0.743(0.077) | 0.748(0.078) | 0.04 | 1/42 | 0.840 |
| T. Ca (mM) | 2.161 (0.159) | 2.222 (0.153) | 1.55 | 1/42 | 0.219 |
| T. Ca / T. Mg | 2.936 (0.0351) | 3.001 (0.424) | 0.52 | 1/42 | 0.475 |
| EMM Mg (mM) | 0.739 (0.020) | 0.750 (0.015) | 0.17 | 1/41 | 0.682 |
| EMM Ca (mM) | 2.177 (0.039) | 2.213 (0.029) | 0.55 | 1/41 | 0.462 |
| Albumin (g/l) | 44.21(3.72) | 42.21(3.36) | 3.32 | 1/42 | 0.075 |

All data are shown as means (SD), except the EMM data

T. Mg: Total magnesium; T. Ca: Total Calcium; T. Mg: Ionized magnesium

*EMM (SE): estimated marginal means after adjusting for albumin

of changes in calcium-sensing receptors (Bohrer and Krannich 2007; De Baaij et al. 2015). Secondly, Mg deficiency may dysregulate neuronal functions including N-methyl-D-aspartate (NMDA) channels, leading to depression (Derom et al. 2013; Jung et al. 2010; Eby et al. 2011). Thirdly, long-term Mg insufficiency may cause activated immune-inflammatory pathways because Mg has anti-inflammatory effects through suppression of nuclear factor-κB (Weglicki et al. 1992; Elovainio et al. 2006; Sugimoto et al. 2012).

Serum total and unbound Ca were significantly lower in MDD than in controls. These findings extend those of previous authors (Bowden et al. 1988; Islam et al. 2018). Jung et al. (2010) and Paul (2001) proposed that lowered Ca levels in the peripheral blood and abnormal neuronal calcium homeostasis may cause depressive symptoms. Not only extracellular, but also intracellular, Ca levels play a role in mood and cognition through effects on neuronal signaling pathways modulating neuroplasticity and cognitive functions including learning (Toescu and Verkhratsky 2007; Hurst 2010; Grützner et al. 2018), while the calcium signaling pathway play a role in long-term depression and memory formation (Mulkey et al. 1994; Lisman et al. 2002). Nevertheless, some authors found that the incidence of hypercalcemia was elevated in depressed patients as compared with controls (Levine et al. 1999) while other studies found that a higher serum Ca/Mg ratio may be associated with the risk to develop depression (Jung et al. 2010). Mg plays a role in the metabolism of vitamin D and Ca balance (Deng et al. 2013) and dysregulations in the balance between Mg and Ca participate in the pathophysiology of depression (Dittman et al. 2000; Anglin et al. 2013).

**Table 5** Effects of treatments on severity of depression and biomarkers

| Dependent variable | Time | | | | | Time x treatment | | |
|---|---|---|---|---|---|---|---|---|
| | $t_0$ | t end-point | W | df | p | W | df | p |
| BDI-II | 49.38 (1.41) | 16.56 (1.06) | 356.89 | 1 | <0.001 | 9.71 | 2 | 0.002 |
| T. Mg (mM) | 0.753 (0.012) | 0.687 (0.029) | 3.98 | 1 | 0.046 | 0.64 | 2 | 0.727 |
| T. Ca (mM) | 2.176 (0.024) | 2.328 (0.035) | 14.19 | 1 | <0.001 | 5.05 | 2 | 0.080 |
| residualized Mg | 0.344 (0.087) | −0.170 (0.211) | 4.33 | 1 | 0.038 | 0.61 | 2 | 0.736 |
| residualized Ca | −0.354 (0.115) | 0.272 (0.181) | 9.54 | 1 | 0.002 | 3.18 | 2 | 0.204 |

All results of generalized estimating equation analyses, repeated measurements, examining the effects of treatments on the Beck Depression rating scale II (BDI-II) score and biomarkers

Time: effects of treatment on the dependent variables; time X treatment: effects of sertraline alone versus sertraline *plus* ketoprofen

Data are shown as mean (SE)

T. Mg: Total magnesium; T. Ca: Total Calcium

Residualized: residual valued obtained by regression on albumin levels

**Table 6** Associations between diverse biomarkers and the Beck Depression Inventory rating scale II (BDI-II) score and an index of immune activation

| # GEE | Dependent variables | Explanatory variables | B | SE | W | df | P |
|---|---|---|---|---|---|---|---|
| #1 | BDI-II | T.Mg | 0.044 | 0.111 | 0.16 | 1 | 0.690 |
| #2 | BDI-II | T.Ca | −0.162 | 0.063 | 6.67 | 1 | 0.010 |
| #3 | BDI-II | Residualized Ca | −0.105 | 0.064 | 2.66 | 1 | 0.103 |
| #4 | BDI-II | Residualized Ca | −0.147 | 0.065 | 5.09 | 1 | 0.024 |
| | | M1 + Th1 + Th2 + Treg | 0.329 | 0.053 | 38.28 | 1 | <0.001 |
| | | Zinc | −0.177 | 0.043 | 16.77 | 1 | <0.001 |
| | | IDO | 0.257 | 0.046 | 31.93 | 1 | <0.001 |
| #5 | M1 + Th1 + Th2 + Treg | Residualized Ca | −0.207 | 0.087 | 5.62 | 1 | 0.018 |

All results of generalized estimating equation (GEE) analyses, repeated measurements

BDI-II: Beck Depression Inventory score

T.Mg: total magnesium; T.Ca: total calcium; Residualized Ca: the residualized Calcium values obtained after regression on albumin

M1 + Th1 + Th2 + Treg: immune activation index; computed as z value of IL-1 (zIL-1) + zIL-6 + zIL-18 + z interferon (IFN)-γ + zIL-4 + z transforming growth factor (TGF)-β1

In the current study, we could not observe a significant relationship between baseline Mg and Ca levels and severity of illness. Previously, it was shown that severity of depression was significantly associated with serum levels of Mg (Styczeń et al. 2015; Rajizadeh et al. 2016), while another study could not detect significant correlations between severity of illness and serum Mg in MDD (Ram et al. 2016). Previous data show that, in MDD, serum total Ca was significantly associated with neuropsychological scores, including executive functions, processing speed and global assessment (Sharma et al. 2017). However, the study of Ram et al. (2016) found no correlations between the severity of depressive symptoms and serum Ca levels (Ram et al. 2016).

The significant positive correlation between albumin and total Mg detected in our study may be explained by the knowledge that a large part of Mg (around 25%) is bound to albumin (Kroll and Elin 1985). Surprisingly, we found highly significant associations between albumin and ionized Ca and the corrected Ca index indicating that those "corrected" values (Ohbal et al. 2014; Mateu-de Antonio 2016) cannot be applied to our population. Therefore, we have adjusted our total Ca and Mg data for albumin using regression analysis and used the residualized values as indices of unbound Ca and Mg, respectively. As such, we found that the unbound (residualized) Ca and Mg values were significantly lower in MDD patients as compared with controls. We found no significant relationships among serum total Mg and Ca levels. Previously, a strong correlation between ionized Ca and Mg has been observed (Ryden et al. 1976) reflecting a close physiologic relationship between these divalent cations in serum (Altman 1977).

## Effects of treatments on Ca and Mg

The second major finding is that antidepressants (with or without ketoprofen) significantly elevated total Ca and that the changes over time in Ca were significantly and inversely associated with improvements in the BDI-II score. Moreover, the effects of increased unbound Ca levels on the severity of illness remained significant after adjusting for the effects of immune activation, and IDO (both positively related with the BDI score), and zinc (inversely related with the BDI score). Elevated production of IFN-γ and M1 macrophagic cytokines may lead to activation of peripheral and brain levels of IDO (Maes et al. 1994; 2011b), while lowered levels of zinc show intertwined associations with immune activation (Twayej et al. 2019). Importantly, in the present study, we found that unbound Ca was significantly and inversely associated with the immune activation index. Previously it was shown that the metabolism and processing of cytokines is calcium-dependent and that calcium signaling plays a key role in diverse immune responses (Ainscough et al. 2015; Fracchia et al. 2013). These data suggest that lowered Ca levels may contribute to immune activation in MDD and that intertwined associations between calcium, zinc and immune activation participate in the pathophysiology of MDD.

In the current study, we found that antidepressant treatments had a weak albeit suppressant effect on total and unbound Mg values. Previously, contradictory findings were reported. Camardese et al. (2012) reported that Mg levels correlated with the patient's responses to antidepressant treatment, whilst Young et al. (1996) found no significant differences in Mg levels or Ca/Mg ratio in drug-free MDD patients compared with control group independently of responsiveness to the antidepressant treatment. In the remission phase, Mg levels were not significantly different from the concentrations of the healthy controls (Styczeń et al. 2015), suggesting that lowered Mg levels during the acute phase may be a state marker of depression. Nevertheless, the suppressant effect of antidepressants on Mg levels may be another side effect of these type of drugs. For example,

some antidepressants may induce diarrhea and sweating, which could induce lowered Mg levels (DiNicolantonio et al. 2018). Since increased Ca intake or Ca excess may worsen Mg deficiency (DiNicolantonio et al. 2018), it is also possible that the increased levels of Ca following antidepressant treatments may have caused a decrease in Mg levels. Although there are well-known effects of Mg on the development of systemic immune responses (Moslehi et al. 2012), the current study could not find a significant association between Mg levels and the immune activation index. Nevertheless, extracellular Mg concentrations may not reflect their intracellular levels and therefore the current assays of Mg status may not be satisfactory to reflect intracellular Mg (Serefko et al. 2016).

## Conclusions

Baseline serum total and ionized Mg and Ca were significantly lower in MDD as compared with controls but were not associated with severity of illness. Treatment with antidepressants (with or without ketoprofen) significantly lowered serum Mg and elevated Ca levels. The increments in the latter were significantly associated with the clinical efficacy of antidepressants. The results suggest that lowered Mg and Ca levels contribute to the pathophysiology of MDD, lowered Ca contributes to immune activation, and intertwined associations between calcium, zinc and immune activation participate in the pathophysiology of MDD and the clinical response to antidepressants.

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## Compliance with ethical standards

**Conflict of interest** The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

**Abbreviations** *ANOVA,* Analysis of variance; *BDI-II,* Beck Depression Inventory-II; *BMI,* Body mass index; *Ca,* Calcium; *CIRS,* Compensatory immune regulatory system; *COX,* Cyclooxygenase; *CRP,* C-reactive protein; *CV,* Coefficient of variation; *ELISA,* Enzyme-linked immunosorbent assay; *GEE,* Generalized estimating equation; *GLM,* General linear model; *HRP,* Horseradish peroxidase; *I,* Ionized; *ICD-10,* 10th revision of the International Statistical Classification of Diseases and Related Health Problems; *IDO,* Indoleamine 2,3-dioxygenase; *IFN-γ,* Interferon-gamma; *IL,* Interleukin; *IRS,* Immune response system; *M1,* Macrophage type 1; *MDD,* Major depressive disorder; *Mg,* Magnesium; *NSAIDs,* Non-steroidal anti-inflammatory drugs; *T,* Total; *TGF-β1,* Transforming growth factor-beta1; *Th-,* T helper-; *TMB,* Tetramethylbenzidine; *Treg,* Tregulatory cells

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