Reports of Biochemistry & Molecular Biology
Vol.7, No.2, Jan 2019

Original article

www.RBMB.net

Increased Levels of Acetylcholinesterase, Paraoxonase 1, and Copper in Patients with Moderate Depression- a Preliminary Study

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Abstract

Background: Depression is a common and widespread mood disorder, which affects an emotional level that varies widely in its intensity. Biochemical parameter alterations have been observed in different depression types. In the present study, we examined acetylcholinesterase (AChE), paraoxonase 1 (PON1), and copper levels in moderately-depressed patients and healthy controls to ascertain whether the measurement of red blood cell (RBC) AChE, and plasma PON1 and copper could be used to evaluate moderate depression.

Methods: This case control study was performed in the Department of Biochemistry, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India. Patients who met ICD 10 diagnostic criteria were considered as cases. Goldberg's General Health Questionnaire 28 (GHQ-28) was used to select controls. Four ml of blood was collected from 24 cases and 20 controls aged 35-70 years and used to determine RBC AChE, and plasma PON1 and copper levels.

Results: Red blood cell AChE, and plasma PON1 and copper levels were significantly greater in patients with moderate depression than in controls. Further, a receiver operating characteristic curve for validity of the biochemical parameters in plasma from patients with moderate depression indicated sensitivity and specificity above 85% for copper and PON1.

Conclusions: Red blood cell AChE, plasma PON1, and copper levels may have roles in the pathogenesis of depressive disorders.

Keywords: Acetylcholinesterase, Copper, Moderate Depression, PON1, Stress.

Introduction

Depression is a common and widespread mood disorder, which affects an emotional level that varies widely in its intensity. It leads to distorted thinking, judgment, and decision-making and makes it difficult to remember the last time one felt "normal" and hard to feel "normal" again (1). It manifests as a combination of feelings of hopelessness, loneliness, sadness, irritability, worthlessness, guilt, and agitation, accompanied by range of physical symptoms. The degree of suffering associated with depression is comparable to that experienced in in most chronic medical conditions (2). The prevalence of depression in the general population ranges from 10 to 15% (3). Indian statistics reported an overall prevalence of 15.9% for depression. The World Health Organization (WHO) estimates that 350 million people globally suffer from depression, and if this trend continues, it will be the leading cause of disease burden by 2030 (4).

Various hypotheses have been proposed to

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Received: 12 Jun, 2018; Accepted: 17 Jul, 2018

explain the etiology of depression through the neuro-oxidative theory of depression. Damage to DNA, mitochondria, proteins, and lipids have been reported in major depressive and bipolar disorders due to heightened nitro-oxidative and nitrosative damage. These results suggest that the activation of neuro-oxidative and neuro-nitrosative pathways play key roles in major depressive disorders (5, 6). Major depressive disorder is commonly accompanied by a decrease in antioxidant levels, increased susceptibility to lipid peroxidation (7) and increased malondialdehyde (MDA) levels (8).

Previous studies have indicated that acetylcholinesterase (AChE) inhibitor increases acetylcholine levels, which has been thought to decrease manic symptoms and improve depressive symptoms (9); however, more recent data indicated no association between AChE inhibitors and anxiety and depression symptoms in elderly adults with dementia (10).

Paraoxonase 1 (PON1) is synthesized in the liver and transported along with high-density lipoproteins (HDL). It has been studied in various disorders and stressful events and hypothesised to be an effective antioxidant (11). Studies have indicated that low HDL levels are associated with depression (12) and increase endothelial dysfunction, which is responsible for higher atherogenic index and greater risk of mortality from coronary artery disease (CAD) in depression (13). Paraoxonase 1 prevents HDL and LDL oxidation via its antioxidant property (14); thus, lower HDL levels can lead to decreased PON1 activity in depression.

Copper is an integral part of many enzymes including dopamine hydroxylase, catalase, cytochrome oxidase, superoxide dismutase, and others, and is an essential trace element affecting many biochemical functions. Psychiatric disturbances have been reported in patients with disorders of trace elements (15). Monoamine oxidase is also a copper-dependent enzyme and is affected in major depressive disorder, alcoholism, and psychiatric disorders (16); therefore, we hypothesize that copper has an integral role in deranging the biochemical profile in patients with moderate depression.

The primary objective of this study was to measure AChE, PON1, and Cu levels in moderately-depressed patients and healthy controls and determine whether these values could predict moderate depression.

Materials and methods

Twenty-four patients (14 women and 10 men) who met the International Statistical Classification of Diseases and Related Health Problems (ICD)-10 diagnostic criteria for depression (17) and 20 healthy controls (10 women and 10 men) were recruited for the case–control study. The patient group consisted of 24 patients aged 35–70 years (mean age 48.85 ± 6.73) with moderate depression who visited Dr. A. V. Baliga Memorial Hospital, Udupi, between August 2015 and December 2017. All the participants were from Udupi district, coastal Karnataka, South India. Healthy participants, belonging to the same socioeconomic status as the patients, were grouped as controls (aged 35-70, mean age 47.54 ± 8.16). Informed consent was obtained from all the participants.

Inclusion Criteria

Patients who met the ICD 10 diagnostic criteria and newly diagnosed with depression associated with or without neurotic symptoms and who were not on any prior anti-depressant treatment were included. Control subjects were recruited through advertisement on the hospital notice board or through referral by hospital staff. The control subjects were included in the study only if they scored less than 4 major points or 14 minor points on Goldberg's General Health Questionnaire 28 (18).

Exclusion criteria

Patients suffering from depression with psychotic symptoms, psychotic disorders including schizophrenia, schizoaffective disorder, bipolar disorder, severe personality disorder, or on any long-term medications for any chronic diseases, alcoholics, and smokers were excluded. Those suffering from chronic disorders including diabetes mellitus, tuberculosis, acquired immune deficiency syndrome (AIDS), autoimmune disorders, thyroid disorders, renal or liver diseases, cerebro-vascular accident, or seizures were also excluded.

This study was approved by Institutional Ethics Committee (decision number 66/2015; 10.02.2015) for human research. Participants were included in the study only after obtaining informed consent from the legal guardians for participation. All

procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Blood collection and processing

After consent, under tourniquet pressure and aseptic precautions, 4 ml of blood was collected in lithium heparin vacutainers. Blood was collected after the patient was admitted to the ward but before the administration of any medication for depression or associated mood disorders.

Samples were centrifuged at 700 RCF for 15 min, Plasma was separated, transferred to Eppendorf tubes, and used to determine of PON1 and copper levels.

PON1 was measured spectrophotometrically by the method described elsewhere, with minimal modifications. (19) Briefly, the assay mixture contained 500 µl of 2.2 mmol/l paraoxone substrate in 0.1 mol/l Tris-HCl buffer, ph 8.0, containing 2 mmol/l CaCl2 and 50 µl of fresh plasma. The absorbance was monitored at 405 nm at 25 °C. The PON1 activity was expressed in international units (IU). One IU was defined as 1 µmol of p-nitrophenol which was formed/min/L at 25 °C.

Copper was measured using 3,5- dibromo-2-pyridylazon-ethyl -N-3 sulphopropyl aniline. (20)

For RBC AChE, the separated blood cells were washed three times with saline and then 200 µl of the washed cells were haemolysed by diluting with distilled water (1:50). 20 µl of the hemolysate was assayed for AChE. AChE catalyzes the hydrolysis of acetylthiocholine to thiocholine and acetate. The thiocholine production rate was measured by the reaction of thiocholine with 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB). The rate of formation of yellow anion was measured at 410 nm at 25 °C in a thermostated cuvette. Activity was expressed as µmol of substrate hydrolysed at 25 °C/g of hemoglobin (Hb). (21)

A receiver operating characteristic (ROC) curve is a measure of sensitivity versus specificity of the diagnostic test. The cut-off points on the curve are used to determine whether the test results are positive. ROC analysis was performed to assess the validity of the above parameters over the range of possible points for the predictor variable.

Statistical analysis

Statistics were analyzed using Statistical Package for Social Sciences, version 15.0 (SPSS Inc. South Asia, Bangalore). The data for AChE, plasma PON1, and copper were not normally distributed; hence, the Mann-Whitney U test was used for the analysis. Spearman's correlation coefficient (r) was used to determine the association between the variables. P values <0.05 were considered statistically significant.

Results

AChE, PON1, and copper levels were significantly greater in patients with moderate depression than in controls (P<0.001) (Table 1).

A ROC curve for validity of the biochemical parameters in plasma of patients with moderate depression is shown in Table 2. The sensitivities and specificities for copper and PON1 were 91.7 and 90% and 87.5 and 85%, respectively. However, AChE exhibited 70.8 and 95% sensitivity and specificity.

Table 1. RBC acetylcholinesterase and plasma PON1 and copper levels in cases and controls.

Parameters	Controls (n=20)	Moderate depression (n=24)
	Median (IQR)	
Acetylcholinesterase (µmol /g of Hb)	165.06 (129.46,188.10)	249.37 (170.19, 299.73) *
Paraoxonase 1 (nmols/ml/min)	10.00 (6.47, 12.95)	26.20 (20.00, 43.07) *
Copper (mg/dl)	26.80 (20.50, 79.07)	167.40 (125.67,208.50) *

Values expressed as Median IQR (Interquartile range).
*p<0.001 using Mann-Whitney U test.

AChE was weakly correlated with PON1 in female patients with moderate depression. No other parameters were significantly correlated based on gender (Table 3). The overall correlations between the parameters are shown in Table 4. PON1 was weakly correlated with copper levels in patients with moderate depression.

Table 2. Validity of the biochemical parameters in patients with moderate depression.

Parameters	Optimal cut off using ROC	Area under the curve (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Acetylcholinesterase (µmol /g of Hb)	≥ 214.3	0.804	70.8	95	94.4	73.1
Paraoxonase 1 (nmols/ml/min)	≥ 18.2	0.918	87.5	85	87.5	85
Copper(mg/dL)	≥ 103.4	0.973	91.7	90	91.7	90.0

Sensitivity and specificity above 85% for copper and PON1

Table 3. Correlation coefficient between AChE, PON1, and Cu in patients with moderate depression based on gender.

Parameters	Male		Female	
	Spearman correlation (r)	P value	Spearman correlation (r)	P value
AChE (µmol /g of Hb) with PON1 (nmols/ml/min)	-0.188	0.603	-0.488	0.077
AChE (µmol /g of Hb) with Cu (mg/dl)	-0.449	0.193	-0.299	0.299
PON1 (nmols/ml/min) with Cu (mg/dl)	0.418	0.230	0.283	0.327

P<0.05 was considered statistically significant.

Table 4. Correlation coefficient between AChE, PON1, and Cu in patients with moderate depression.

Parameters	Spearman correlation (r)	P value
AChE (µmol /g of Hb) with PON1 (nmols/ml/min)	-0.257	0.225
AChE (µmol /g of Hb) with Cu (mg/dl)	-0.277	0.191
PON1 (nmols/ml/min) with Cu (mg/dl)	0.349	0.094

P<0.05 was considered statistically significant.

Discussion

Depression is a common mental disorder that adversely affects quality of life and cognitive function; it also increases morbidity and mortality. The pathophysiology of depression is multifactorial with decrease in monoamines and brain-derived neurotrophic factor (BDNF), alterations in neurotransmitter receptors, and abnormalities in hypothalamic-pituitary-adrenal (HPA) axis. In addition, a number of studies have reported that oxidative stress can play a role in the pathogenesis of depression (22).

The present findings demonstrate that AChE, PON1, and copper levels are greater in depressed patients than in controls.

Acetylcholinesterase decreases in blood and brain (23) during stress. Jope et al. indicated that the cholinergic system follows a unique pattern of differences in diverse psychiatric conditions. They showed that the RBC AChE decreased in depression and schizophrenia, but was unchanged in manic patients (24). Our study contradicts some previous reports and indicated greater AChE levels

in patients with moderate depression. This study agrees with the study of Tiwari et al., which indicated that AChE activity is increased in depression patients, and this might be a compensatory mechanism employed by the body to counteract the excess of acetylcholine in the body (25).

In this study plasma PON1 was significantly greater in patients with moderate depression than in healthy controls. This was contrary to a previous report stating that PON1 decreases in depression (26). PON1 acts as an endogenous free radical scavenger and protects high- and low-density lipoproteins (HDL and LDL) from oxidation (14). It is associated with oxidative stress, and increased stress results in decreased levels of PON1 due to utilization of the antioxidant power to neutralize the free radicals and oxidants (27).

Ari et al. reported a reduction in PON1 in major depressive disorders and supported that underlying oxidative stress in depression might be the cause of the enzyme's reduced activity (28). All the above results are based on western studies, therefore in Indian context the results might vary due to the multifactorial nature of the enzyme, cultural differences, different food habits, dietary changes, or the presence of unusual polymorphisms.

Copper is an essential mineral found ubiquitously in food and drinking water. The level in blood can increase due to excess intake or inadequate removal by the liver. Use of copper water pipes and/or copper compounds in water to prevent fungus and algae growth are major source of excess copper exposure (29). Stress also causes copper accumulation. Zinc, being the primary antagonist of copper, decreases in stress and causes adrenal fatigue. A fatigued adrenal gland fails to produce sufficient hormones to promote the hepatic synthesis of ceruloplasmin. Adrenal fatigue and ceruplasmin deficiency result in copper accumulation in the body, (30) leading to anxiety, stress, and depression (31). In agreement with previous studies, in this study copper was significantly greater in patients with moderate depression than in controls.

Referring to Table 3 and 4, AChE was negatively correlated with PON1 and copper while PON1 was positively correlated with copper levels. Although none of these correlations were statistically significant, the correlations indicate an association between the studied parameters; a study with a larger sample size might give a significant association.

In this study AChE, PON1, and copper were increased in moderate depression, and oxidative stress is one of the underlying causes associated with the derangement of those parameters. Thus, the intervention of effective stress-coping strategies may balance these altered parameters and improve the quality of life in moderately-depressed patients. Further, the ROC curve of the biochemical parameters in plasma of patients with moderate depression indicate that PON1 and copper have sensitivities and specificities of 85% and above.

Our study indicates that due to heightened oxidative stress, AChE and plasma PON1 levels were increased in moderately-depressed patients. The increase in AChE is a compensatory mechanism to decrease the high acetylcholine levels. Although decreased PON1 is characteristic of depression, we found PON1 to be increased, which might be due to geographical differences or differences in dietary habits of our south Indian subjects. Copper excess and faulty copper removal due to stress results in copper toxicity, anxiety, and depression. Dietary antioxidant therapy, yoga, exercise, and music can effectively combat the stress-causing oxidants and provide a healthy lifestyle to the depressed individual. Further research is needed to explore PON1 polymorphisms in the Indian population, the role of micro minerals in depression patients using a larger sample size.

The small sample size is a limitation to our study. To verify the efficacy of the above parameters in diagnosis, the sample size is inadequate and a limitation in external validity.

Acknowledgment

Funding: This study was funded by Indian Council of Medical Research (ICMR) – Short Term Studentship grant (grant number: 2015-03528).

Mr. Devesh Bhaskar Yerrapragada received a Short-Term Studentship grant from the Indian Council of Medical Research (ICMR). We declare that there is no conflict of interest.

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