Elevated plasma homocysteine in association with decreased vitamin B_{12}, folate, serotonin, lipids and lipoproteins in depressed patients

MO Ebesunun[1], HU Eruvulobi[1], T Olagunju[1], OA Owoeye[2]
[1]Chemical Pathology, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Sagamu, Nigeria
[2]Federal Neuropsychiatric Hospital, Yaba, Lagos, Nigeria

Abstract
Objective: Increased plasma homocysteine, decreased vitamin B_{12} and folic acid levels have been implicated in depressive mood. Plasma homocystine, vitamin B_{12}, folic acid tryptophan, lipids and lipoproteins were determined in depressed patients and controls. **Method:** Sixty subjects consisting of 30 depressed patients and 30 apparently healthy volunteers, who served as controls, were selected for this study. Anthropometric indices and biochemical parameters were determined using standard procedures. **Results:** The results showed a significantly higher plasma homocysteine level amongst depressed patients when compared with the corresponding controls ($p<0.001$), the percentage increase was 116%, while the plasma vitamin B_{12} ($p<0.01$), total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol levels ($p<0.001$) were markedly lower when amongst depressed patients when compared with the corresponding controls; the percentage differences were 21%, 42% and 42% respectively. Plasma triglyceride, folic acid and tryptophan levels amongst depressed patients were not significantly different from the controls. The male subjects had significantly higher plasma tHcy levels than the female counterparts ($p<0.001$). **Conclusion:** This study showed a significant increase in plasma tHcy coexisting with a decrease in plasma vitamin B_{12} TC, LDLC and HDLC, in depressed patients. Increased plasma homocysteine could be a sensitive indicator of plasma B vitamin deficiency.

Keywords: Cholesterol; Depression; Homocysteine; Tryptophan; Vitamins

Received: 16-11-2010
Accepted: 06-04-2011
doi: http://dx.doi.org/10.4314/ajpsy.v15i1.3

Introduction

There is increasing speculation that diet has an important impact on mental health and that the outcomes of certain mental health disorders, including depression may be influenced by nutritional factors.[1] Although considerable research has focused on understanding the biochemistry and assessing the efficacy of pharmaceutical interventions for depression[2], it is only recently that research attention has focused on the impact of diet.

Correspondence
Dr MO Ebesunun
Chemical Pathology, Obafemi Awolowo College of Health Sciences
Olabisi Onabanjo University, Sagamu, Nigeria
email: onoebe@yahoo.com

Emerging evidence suggests that certain nutrients may be important in the pathogenesis and treatment of depression.[3] Reports from studies[4,5] have shown that naturally occurring low total cholesterol levels, specifically levels below 4.14mmol/L (160mg/dl), as well as low density lipoprotein cholesterol (LDLC) and triglyceride concentration are associated with trait measures of depression and anxiety.[4] An explanation for this is that low cholesterol concentration causes changes in the cholesterol content of the synaptosomal membrane and a decrease in the number of serotonin receptors which are essential in mood regulation.[5]

Recent studies have also shown that high total plasma homocysteine is a sensitive marker of functional deficiency of either folic acid or vitamin B_{12}.[6,7] High levels of homocysteine have being associated with cerebrovascular disease, monoamine neurotransmitters and depression of mood.[7] A

plausible hypothesis for these associations is that high homocysteine levels cause cerebral vascular disease and neurotransmitter deficiency, which could cause depressed mood.[8]

Available evidence indicates that homocysteine is directly toxic to neurons and blood vessels and can induce DNA strand breakage, oxidative stress, and apoptosis.[8] The methionine-homocysteine metabolic pathway intermediaries, S-adenosylmethionine and S-adenosylhomocysteine, produce methyl groups required for the synthesis of catecholamines and DNA.[9] Since homocysteine is a sensitive indicator of B vitamin deficiency, an elevated homocysteine level has been suggested as a marker for a pathogenic process.[9]

Hyperhomocysteinemia has been associated with depression in different populations, but there is paucity of information on the relationship between homocysteine and depression in Nigeria where the diet is mainly carbohydrate based and low in vitamin nutrients.

Similarly, studies involving tryptophan, a serotonin precursor, indicated that low plasma serotonin levels were associated with depression.[10,11] A dietary deficiency of tryptophan has being shown to aggravate depression.11

The current study set out to ascertain whether elevated plasma homocysteine exists in samples of patients and controls with/without depression and if it does, does it coexist with decreased plasma vitamin B_{12} and /or folic acid.

Methods

Subjects

The subjects for the study consisted of 30 patients (9 males, 21 females) with major depression as diagnosed by the attending consultant psychiatrist and met the ICD-10 diagnostic criteria for severe depressive episode. The clinical assessment and laboratory investigations did not reveal any underlying or co-morbid chronic medical conditions, infections or alcohol abuse. They were randomly selected out of 100 patients at two locations. The patients were aged between 23 and 63 years with a mean age of 37.9 ± 2.1 years. Patients with evidence of cognitive impairment, severe physical illness, or drug or alcohol misuse, cancer, tuberculosis, renal dysfunction and HIV patients were excluded. Also excluded were those with previous history of hypertension, diabetes and cardiovascular diseases.

Thirty apparently healthy volunteers with a mean age of 42.50 ± 1.5 were included as controls. These were randomly selected from 70 volunteers and were medically assessed and not suffering from depression as assessed by the consultant psychiatrists..

Ethical approval was obtained from the Medical Ethical Committee of the Federal Neuro-Psychiatric Hospitals Lagos and Abeokuta Nigeria. The patients were capable of making written or oral informed consent. Written/oral informed consent was obtained from every participant.

Anthropometric assessment

The weight in kilogrammes was measured using a weighing scale (Seca GMBH co. Germany). Height in centimeters was taken with the subjects in a standing position without footwear using a measuring rod attached to a fixed scale. This measured to an accuracy of 0.1 centimeter. The body mass index in kg/m^2 was calculated using weight/height^2.[12]

Blood sample collection

Blood samples were drawn in the morning after an overnight fast of about 10-14 hours into EDTA bottles. They were immediately placed on ice and separated within 2 hours by centrifugation. The plasma samples were stored at -20°C until analyzed for homocysteine, vitamin B_{12}, folic acid, TC, TG, HDLC and tryptophan.

Methods

The plasma homocysteine concentration was estimated using Enzyme Immunoassay (EIA) method[13] reagents was supplied by Axis-Shield Diagnostics Limited (United Kingdom).

The plasma total cholesterol concentration was estimated using enzymatic colorimetric method. It was determined using a single aqueous reagent supplied by Randox® (Randox Laboratories Limited, United Kingdom) as described by Allain et al.[14]

The plasma triglyceride was estimated using an enzymatic hydrolysis method.[15] The single aqueous reagent was also supplied by Randox. The high-density lipoprotein cholesterol (HDLC) was determined with the use of a modified phosphotungstic acid/$MgCl_2$ precipitation procedure for the precipitation of apolipoprotein B- containing lipoproteins. The HDLC in the supernatant was then determined using the cholesterol oxidase-peroxidase method.[15] LDLC was calculated using Friedwald formula.[16] Vitamin B_{12}, folic acid and tryptophan were analysed using high power liquid chromatography (HPLC) Waters 616/626.

Accuracy and precision of biochemical tests were monitored by including commercial quality control samples within each batch of test assay.

Statistical analysis

All results were subjected to statistical analysis using the SPSS software. The difference between means was assessed using a Student t-test. The results were regarded as significant at $p<0.05$.

Results

Table I shows the age and physical parameters in depressed and control subjects (Mean ± SEM). There were no significant differences in any of the parameters when comparing patients with the corresponding control values.

Table I: Age and physical parameters in depressed subjects and controls (Mean ± SEM)

Variable	*Depressed Subjects* (n = 30)	*Controls* (n = 30)	*t-value*	*p-value*
Age (yrs)	37.9 ± 2	42.5 ± 1	-1.726	NS
Weight (kg)	56.2 ± 2	59.9 ± 2	-1.693	NS
Height (m)	1.6 ± 0	1.6 ± 0	-0.271	NS
BMI (kg/m^2)	21.5 ± 1	22.9± 0.5	-1.633	NS

SEM = Standard Error of Mean
BMI = Body Mass Index
NS = Not Significant

Table II: Biochemical parameters in depressed patients and controls (mean ±SEM)

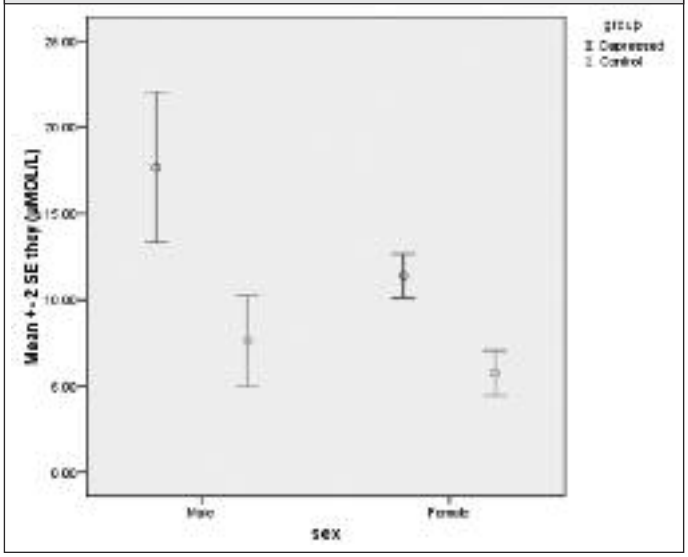
Variables	*Patients N=30*	*Controls N=30*	*t-value*	*p-value*	*% difference*
TC (mg/dl)	124.35 ± 4.56	176.39 ± 4.66	-7.982	p< 0.001	42↓
TG (mg/dl)	64.59 ± 4.94	79.66 ± 6.62	-1.825	ns	23↓
HDLC (mg/dl)	41.15 ± 2.62	58.79 ± 1.91	- 5.434	p< 0.001	42↓
LDLC (mg/dl)	70.31 ± 4.89	102.19 ± 4.71	-4.691	p< 0.001	46↓
HDLC/TC	0.56 ± 0.03	0.57 ± 0.02	-0.585	ns	1.8↓
LDLC/HDLC	2.03 ± 0.21	1.82 ± 0.11	-0.8860	ns	7.5↓
tHcy (µmol/L)	13.27 ± 0.95	6.36 ± 0.63	6.096	p< 0.001	116↑
Vitamin B_{12} (µg/dl)	38.07 ± 7.62	46.09 ± 5.76	-4.60	p<0.001	21↓
Folic acud (µg/dl)	102.04 ± 9.51	106.51 ± 10.88	-1.69	ns	3.9↓
Tryptophan (µg/dl)	63.93 ± 13.73	68.03 ± 8.72	-1.14	ns	7.9↓

SEM = Standard Error of Mean
TC = Total Cholesterol
TG = Triglyceride
HDL-C = High Density Lipoprotein Cholesterol
LDL-C = Low Density Lipoprotein Cholesterol
tHcy = Total plasma Homocysteine
ns = Not Significant.

Table II shows the biochemical parameters in the depressed and control subjects (mean ± SEM). There were significantly lower levels in the plasma of TC (42%), HDLC (23%), LDLC (46%), ($p < 0.001$) and vitamin B_{12} (21%) ($p<0.01$) in the depressed patients when compared with the corresponding control values. On the other hand, there was a significantly higher plasma tHcy (116% higher) ($P < 0.001$) when compared with the corresponding control values. No significant differences were noted in the other parameters. There were however 3.9% and 7.9% lower levels in plasma folic acid and tryptophan respectively, amongst depressed patients. When patients were classified into different socioeconomic classes, no significant differences were obtained.

Figure 1 shows an error bar of plasma homocysteine levels in male and female patients as well as controls. The male subjects exhibited higher values in both patients and controls. The plasma homocysteine was significantly higher in the male depressed patients ($p<0.001$) than the female.

Figure 1: Error bar of plasma homocysteine in male and female depressed patients and controls



Discussion

This study shows that patients with depression have elevated plasma homocysteine in association with low levels of vitamin B_{12}. Furthermore we observed a significant relationship with hyperhomocysteine and non significant relationship with folic acid in our depressed patients. This study could not demonstrate whether the observed association with vitamin B_{12} deficiency precedes or results from depression. Lack of appetite is an important feature of depression which could explain this association in part. Therefore, an elevated plasma tHcy concentration, rather than being an independent risk factor, may be an indicator of unhealthy life style, which could lead to an increased risk of depression. Our findings are consistent with studies which showed elevated plasma homocysteine in association with vitamin B_{12} deficiency in depressed patients.[6,7] About 27% of our patients had tHcy greater than 15µmol/L which is a risk factor for the likelihood of premature CVD development.

Patients with depression have been shown to have a twofold to fourfold increased risk of developing CVD[11] either through an increased tHcy, or decreased vitamin B_{12} and folic acid. The mechanism of homocysteine's action in the CNS and its link with depression are unclear, but it is known that homocysteic acid and cysteine sulphinic acid (metabolites of homocysteine) may have an excitotoxic effect on the N-methyl-D-aspartate receptors in the CNS[17] and this in turn may have given rise in part to depression in our patients. This alteration can modify mood, since these metabolites have being reported to function as antidepressants.[7] The modification of mood could also be attributed in part to low plasma vitamin B_{12} as evident from our results, since these vitamins function as cofactors in the

remethylation of homocysteine.

Any disturbance in homocysteine metabolism could lead to decreased methylation, and most likely lead to low levels of neurotransmitters which could cause DNA damage. There was evidence of reduced mean plasma folic acid and tryptophan levels in our depressed patients. Earlier studies[5,17] showed that reduced plasma folic acid can lower the level of serotonin in the brain and this has been associated with depressed mood.

Another plausible explanation is that elevated tHcy observed in our depressed subjects could cause depression by direct neurotoxicity through its effect on the N-methyl-D-aspartate receptors in the CNS. Thus an elevated plasma tHcy may merely be a marker of impaired monoamine metabolism in these subjects, which may have occurred through reduced CNS methylation. Perhaps, the rise in total homocysteine in our patients could be suggesting a failure of methylation of homocysteine to methionine due to reduced availability of the vitamin B_{12} which acts as cofactor for the remethylation reaction.

The males showed a higher plasma homocysteine than the females. Reports from studies elsewhere[10,19,20] have shown higher plasma tHcy in males than females. Our finding supports the reports from previous studies[18,19] that male gender is a risk factor for elevated plasma homocysteine thus making the male more vulnerable to CVD risk.

Reduced plasma TC, LDLC and HDLC were obtained in our depressed subjects. Several explanations have been put forward; one of which is that the relationship may be attributed to changes in the cholesterol content of the synaptosomal membrane and a decrease in the number of serotonin receptors due to a decrease in cholesterol concentration.[4,20,21] According to Engelberg[22] decreased cholesterol concentration in the serum may lower the concentration of membrane cholesterol in the brain, decreased microviscosity and availability of membrane receptors, which in turn may influence the re-uptake of serotonin from the blood, and this results in lower concentrations of serotonin in brain cells, which could be responsible for depressive disorder and aggressive behavior.[4] Loss of appetite which is common with depressed patients may also have been responsible for the low plasma cholesterol. A previous report has shown that low plasma cholesterol is associated with suicide in depressed subjects[6]; theoretically our depressed patients could be more prone to suicidal tendencies.

This study also demonstrated that depressed subjects have significantly lower levels of plasma HDLC than the control subjects. The relationship between plasma HDLC and depression is still not very clear; however, this lower level is regarded as a significant cardiovascular risk. Available evidence has suggested that for every 1mg/dl decrease in plasma HDLC, there is twofold chance of developing CVD risk.[23]

Reports have shown that increased mortality, lower socioeconomic status, cognitive decline, and unsupportive childhood and adult relationships are associated with higher allostatic load scores.[24,25,26,27] When a confounding factor such as socioeconomic status was applied in this study, no positive effect was observed in our depressed patients. Therefore plasma tHcy concentration was not affected by socioeconomic class. In general socioeconomic class did not exert effect on any of the measured parameters. Homocystine is a marker of systemic toxicity, and may contribute to allostatic load in depressed patients.[26]

From the results obtained in this study, it could be speculated that our depressed patients are more likely to be prone to premature CVD risk in the near future.

The young adult patients in this study were all newly admitted and within their first two weeks of admission in their early stage of treatment hence the possibility of the effect of treatment on the outcome of our result if any, would be minimal. Also clinical and laboratory findings of the patients revealed that the patients did not have any associated diseases such as renal dysfunction, CVD , drug or alcohol misuse, cancer, tuberculosis and HIV that could affect the outcome of our findings.

Conclusion

This study has provided evidence for increased plasma tHcy coexisting with decreased vitamin B_{12}, TC, LDLC and HDLC in our depressed patients. Therefore plasma homocysteine and lipid metabolism may play an important role in depression. Whether these changes are causes or effects of depression could not be determined. It could be possible that if vitamin B_{12} is included as a part of the treatment regimens it might be beneficial to depressed patients.

Since there are many causes of elevated plasma homocysteine levels and probably many causes of depression, prospective and intervention studies should include a large sample size to ensure adequate power to demonstrate outcome linked to diet or to other underlying biological factors.

Acknowledgement

We wish to acknowledge the Consultant Psychiatrists, nurses, medical laboratory staff of Psychiatric Hospitals Abeokuta and Yaba Nigeria for their invaluable assistance in the recruitment of the patients. We particularly wish to express our gratitude to Mrs. Doreen Okeke for her assistance in the analysis of homocysteine and Mr. Basil for his assistance in the analysis of the B vitamins and the tryptophan

References

1. *Botez MI, Reynolds EH, eds. Folic acid in neurology, psychiatry and internal medicine. New York: Raven; 1979.*
2. *Bottiglieri T, Crellin R, Reynolds EH. Folate and neuropsychiatry. In: Bailey LB, editor. Folate in health and disease. New York: Marcel Dekker; 1995. 435–462.*
3. *Jacka FN, Kremer PJ, Leslie ER, Berk M, Patton GC, Toumbourou JW, Williams JW. Associations between diet quality and depressed mood in adolescents: results from the Australian Healthy Neighbourhoods Study. Aust NZ J Psychiatry 2010. 44; 435-442.*
4. *Hillbrand M, Spitz RT, VandenBos GR. Investigating the role of lipids in mood, aggression, and schizophrenia. Psychology Update 1997; 48: 7.*
5. *Sheikh N, Farhadi Nasab A, Araghchian M, et al. The relationship*

of cholesterol and triglyceride with depression. Acta Medica Iranica 2000; 42: 185-187.
6. Steegmans PH, Fekkes D, Hoes AW, Bak AA, Vander Does E, Grobbee DE. Low serum cholesterol concentrations and serotonin metabolism in men. BMJ 1996; 312:221.
7. Papakostas GI, Petersen T, Mischoulon D, Green CH, Nierenberg AA, Bottiglieri T, et al. Serum folate: vitamin B_{12}, and homocysteine in major depressive disorder, part 2: predictors of relapse during the continuation phase of pharmacotherapy. J Clin Psychiatry 2004; 65:1096–1098.
8. Tiemeier H, van Tuijl HR, Hofman A, Meijer J, Kiliaan AJ, Breteler MM. Vitamin B_{12}, folate, and homocysteine in depression: the Rotterdam Study. Am J Psychiatry 2002; 159:2099–2101.
9. Folstein M, Liu T, Peter I, Buel J, Arsenault L, Scott T, Qiu WW. The homocysteine hypothesis of depression. Am J Psychiatry 2007; 164:861-867.
10. Bottiglieri T, Laundy M, Crellin R, Toone BK, Carney MW. Reynolds EH Homocysteine, folate, methylation, and monoamine metabolism in depression. J Neurol Neurosurg Psychiatry 2000; 69:228–232.
11. Delgado PL, Charney DS, Price LH, Aghajanian GK, Landis H, Heninger GR. Serotonin function and the mechanism of antidepressant action. Arch Gen Psychiatry 1990; 47: 411–8.
12. Coccaro EF, Siever LJ, Klar HM, Maurer G, Cochrane K, Cooper TB, Mohs RC, Davis KL. Serotonergic studies in patients with affective and personality disorders: correlates with suicidal and impulsive behavior. Arch Gen Psychiatry 1989; 46: 587–99.
13. Jonasson RF, Hedner T, Hultberg B, Ohlin H. Hyperhomocysreinemia is not associated with increased levels of asymmetric dimethylarginine in patients with ischaemic heart disease. Eur J Clin Invest 2003; 33: 543-549.
14. Allain CA, Pooni LS, Cha CA. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20: 470-5.
15. Buccolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem 1973; 19: 476-82.
16. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultra centrifuge. Clin Chem 1972; 18: 499-502.
17. Lipton SA, Kim WK, Choi YB, Kumar S, D'Emilia DM, Rayadu PV, Arnelle DR, Stamler JS. Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. Proc Natl Acad Sci USA 1997; 94: 5923-5928.
18. Kruman II, Culmsee C, Chan SL, Kruman Y, Guo Z, Penix L, Mattson MP. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. J Neurosci. 2000; 20: 6920-6926.
19. Parson RB, Warring RH, Ramsden DB, Williams AC. Invitro effect of cyteine metabolites homocystic acid, homocysteine and cysteic acid upon human neuronal cell lines. Neurotoxicology 1998; 19: 599-604.
20. Levine J, Stahl Z, Ami Sela B, Gavendo S, Ruderman V, Belmaker RH. Elevated homocysteine levels in young male patients with schizophrenia. Am J Psychiatry 2002; 159: 1790-1792.
21. Rabe-JabloÄska J, Poprawska I. Levels of serum total cholesterol and LDL-cholesterol in patient with major depression in acute period and remission. Med Sci Monit 2000; 6: 539-547.
22. Engelberg H. Low serum cholesterol and suicide. Lancet 1992; 339: 727–9.
23. Goldbourt U, Yaani SM, Dale JH. Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality. A 21 year follow up of 8000 men. Atherosclerosis Thromb Vasc Biol 1997; 17: 107-113.
24. Crimmins EM, Johnston M, Hayward M, Seeman T. Age differences in allostatic load: an index of physiological dysregulation. Exp Gerontol 2003; 38: 731–734.
25. Seeman T, Glei D, Goldman N, Weinstein M, Singer B, Yu-Hsuan L. Social relationships and allostatic load in Taiwanese elderly and near elderly. Soc Sci Med 2004; 59: 2245–2257.
26. Singer B, Ryff CD. Hierarchies of life histories and associated health risks. Ann N Y Acad Sci 1999; 896: 96–115.
27. Seeman TE, Singer BH, Ryff CD, Love GD, Levy-Storms L. Social relationships, gender, and allostatic load across two age cohorts. Psychosom Med 2002; 64: 395–406.