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Original Article

Serum cobalamin and red cell folate levels of anti-psychotic treatment and treatment naïve psychiatic patients in a tertiary hospital in Nigeria

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ABSTRACT

Background: Psychiatric disorders contribute significantly to the global burden of diseases. There is an urgent need to curtail the morbidity and mortality associated with psychiatric disorders. Deficiencies of cobalamin and folate have been linked with psychiatric disorders.

Materials and method: Sixty-six each of antipsychotic treatment, treatment naïve psychiatric patients and control were recruited for the study. Red cell folate and serum cobalamin were determined with Enzyme Linked Immunosorbent Assay kits and the haemogram using Sysmex XT2000i.

Result: Folate deficiency was present in 13.6% of newly diagnosed anti-psychotic naive psychiatric patients with few of them having neutrophil hypersegmentation (7.6%) and macrocytosis (4.5%). Mean red cell folate levels for anti-psychotic naïve, patients on anti-psychotic and controls were 350.23 ± 0.54 nmol/l, 370 ± 0.70 nmol/l and 370 ± 0.51 nmol/l respectively, with p-values of 0.0001 and 0.3500 respectively when compared with control, while serum cobalamin levels were within the normal reference range in all patients and controls.

Reticulocyte count had 8 times and 3 times likelihood of influencing low serum folate and low serum cobalamin respectively.

Conclusion: All patients had Serum cobalamin levels within the reference interval, the same can be said of the RBC folate levels of the greater percentage (95.5%) of psychiatric patients on psychotropic drugs.

1. Introduction

Psychiatric disorders contribute significantly to the global burden of diseases.[1] Worst still, projections by experts have shown an expected increase in the burden by 2020.[2] There is therefore an urgent need to curtail the morbidity and mortality associated with psychiatric disorders.

Deficiencies of cobalamin and folate have been linked with dementia, psychosis and other psychiatric disorders.[3–6] Deficiencies of both vitamins and development of psychiatric disorders play causal role to each other.[6] A review study conducted by MA Arroll and co-workers has highlighted a number of possible contributory mechanisms which included oxidative stress, one carbon metabolism (Folate and vitamin B12 metabolism), essential fatty acids insufficiency and immune-mediated responses in the pathophysiology of schizophrenia.[7] Progression to psychiatric disorders due to deficiencies of the vitamins has been attributed to failure of synthesis of S-adenosylmethionine (SAM) due to blockage in the conversion of homocysteine to methionine,[3] while development of deficiencies in the course of psychiatric disorders is due to the use of antipsychotic agents.

For the detection of cobalamin and folate deficiencies, highly sensitive serum methylmalonic acid (MMA) and homocysteine levels would have been more appropriate in the diagnosis of patients with psychiatric disorders. Assay for MMA is complex and the cost of reagents highly exorbitant and not readily available while homocysteinaemia is also found in patients with Chronic Renal Failure,[8] in alcoholism[9] and smoking[10] making its detection not specific for cobalamin and folate deficiencies.

With reduced sensitivity of serum cobalamin notwithstanding, there is still no universally acceptable alternative.[11] Serum folate is also markedly affected by recent diet.[3,11] Red blood cells can store high levels of folate[3] and it is an indicator of tissue folate status since it indicates overall folate turnover in the preceding 2–3 months.[12]

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From these, serum cobalamin and red cell folate levels are sufficient in detecting appreciable number of patients with low levels of vitamins B12 and folate if deficiencies are truly present.

Early detection and correction of deficiencies of these vitamins have been shown to reverse or reduce progression of psychiatric disorders and prevents long term use of antipsychotics.[13] Studies have also shown that the best response to antipsychotic treatment occurs if cobalamin deficiency is reversed within the first 6 months of commencing treatment.[13] Damage becomes irreversible if deficiency is not reversed within 12 months, hence the need for early detection and correction of the deficiencies.[13]

Several studies conducted locally and internationally have only shown conflicting results. Most of the studies reported low levels of both vitamins,[14,15] while others reported low level of red cell folate with high level of serum cobalamin.[16]

The aim of this study was therefore to assess serum cobalamin level and red cell folate of psychiatric patients on antipsychotics and those that are antipsychotic-naïve and compare the results with that of the previous studies. The study aimed at determining the influence of antipsychotics on the genesis and severity of cobalamin and folate deficiencies.

2. Materials and method

Study Area: This study was carried out at the Psychiatric Out-Patient Clinic and Department of Haematology and Blood Transfusion of University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria. UITH is a five star, 504 bedded hospital located at the North Central region of Nigeria. The hospital serves as referral centre for most other hospitals in the region with an estimated population of about 15,450,084[17]

General Adult Psychiatric Clinics are run on Mondays, Tuesdays and Thursdays where patients were reviewed by consultant behavioural scientists.

Study design: it was a cross sectional descriptive case control study.

Study population: consisted of

1. Newly diagnosed, anti-psychiatric naïve patients.
2. Psychiatric patients already on antipsychotics medication on follow-up.
3. Routine blood donors who were certified fit to donate blood and assessed to be free of psychiatric ailments using General health Questionnaire-12 (GHQ-12) served as case controls.

GHQ-12 is a 12 item versions and self administered questionnaire used to screen for psychiatric morbidity. Its use has been validated in this environment, with a cut-off of 3.[18]

Sample technique: a multi-staged technique where all newly presenting antipsychotic-naïve patients who certified inclusion criteria were recruited until the required number was obtained. For those on antipsychotics for follow-up, only those registered with even numbers were recruited for the study. The reason for this was because our data showed that patients on follow-up out-numbered newly presenting ones several folds. Equal number of controls was also recruited for the study.

Inclusion criteria for study population:

1. Adult newly diagnosed antipsychotic naïve psychiatric patients aged 18–65 years who met ICD-10 criteria.[19]
2. Adult psychiatric patients on anti-psychotics who met ICD-10 criteria.[19]

Exclusion criteria for study population:

3. Presence of chronic co-morbidity/ies like hypertension, Diabetes mellitus.
4. Psychiatric patients on haematinics or multivitamins.

Inclusion criteria for controls:

5. Blood donors not on haematinics or multivitamins.
6. Blood donors with no physical morbidity.

Exclusion criteria for controls:

7. Blood donors with GHQ-12 >3.
8. Blood donors who did not give consent.

Sample size: Sixty-six (66) each of the study populations and controls were used based on the formula by AraoyeMO.[20]

Ethical issues: Ethical clearance was obtained from UITH Ethical Research Committee. Written permission was also obtained from Heads of Psychiatry and Haematology departments and Consultants in charge of the patients. A signed informed consent was obtained from every participant before being recruited.

Every participant was given a number to ensure confidentiality. All pieces of information were kept confidential. There was no harm to participants except for slight discomfort during venepuncture. No financial burden on the participants and no punitive measure against those who declined to participate in the study.

Methodology: Eight milliliters of venous blood was obtained aseptically from all patients. Four milliliters each was dispensed into EDTA and plain vacuitainer bottles.

Samples in EDTA bottle: into this sample, 2 ml of 0.2% ascorbic acid[16] was added, thoroughly mixed and stored at −20 °C until analyzed for red cell folate.

Samples in plain bottle: from this sample, sera was obtained by centrifugation and also stored at −70 °C until analyzed for serum cobalamin.

Assay:

1. *Red cell folate*: Diagnostic Automation Folic acid quantitative Enzyme Linked Immuno Sorbent Assay (ELISA) test using Folate ELISA Test Kit was used to estimate red cell folate on every sample.
2. *Serum cobalamin*: Diagnostic Automation vitamin B12 quantitative ELISA test using Vitamin B12 ELISA Test Kit was also used to estimate for serum cobalamin.
3. *Haemogram*: Full Blood Count (MCH, MCHC, MCV, PCV, Platelet count, Reticulocytes count, Reticulocytes index and Total white blood cell count) was determined on every sample using Sysmex XT2000i automated cell counter.

In each case, manufacturer's instruction manual was strictly adhered to.

Data Analysis: data entry and analysis was done using EPI Info version 3.5.1. Results were presented in tabular forms. Mean values of the three groups of respondents were compared and multiple logistic progressions carried out to determine the factors influencing serum cobalamin and red cell folate. A linear relationship between serum cobalamin and red cell folate was also determined.

Statistical significance was tested at a predetermined p-value of <0.05.

3. Results

Socio-demographic pattern: About half (51.5%) and slightly lower (47%) of anti-psychotropic-naïve patients and patients on anti-psychotic respectively were young adults, while slightly more (53%) of patients on antipsychotics were middle aged. Two (3%), each of anti-psychotic naïve patients and controls were above 60 years of age, Table 1.

Male patients (57.6%), predominated the anti-psychotic naïve group while female patients (51.5%) predominated the group on anti-psychotics, Table 2.

Schizophrenia was the diagnosis in the majority of patients, 86.4% and 91% respectively in anti-psychotic naïve and anti-psychotic treatment groups. Other diagnoses were depressive illness (6.1% of anti-psychotic naïve and 4.5% of patients on anti-psychotics), substance use disorder (3% of anti-psychotic naïve and 4.5% of patients on anti-psychotics) and dementia in 1.5% of anti-psychotic naïve patients.

Table 1
Sociodemographic characteristics of respondents.

Variables	Psychotropic Naïve Frequency n (%)	Respondents on psychotropics Frequency n (%)	Control respondents Frequency n (%)
Age group			
18–40	34 (51.5)	31 (47.0)	33 (50.0)
41–60	30 (45.5)	35 (53.0)	31 (47.0)
>60	2 (3.0)	0 (0.0)	2 (3.0)
Sex			
Male	38 (57.6)	32 (48.5)	35 (53.0)
Female	28 (42.4)	34 (51.5)	31 (47.0)

Table 2
Psychiatric diagnoses of participants (Drug-naïve and those on treatment).

Diagnoses	Drug-naïve subjects (%)	Subjects on anti-psychotics (%)
1. Schizophrenia	86.50	91.00
2. Depressive illness	6.10	4.50
3. Substance use disorder	3.00	4.50
4. Mania	3.00	–
5. Dementia	1.50	–

Red cell folate: Nine (13.6%) of the anti-psychotic naïve patients and 3 (4.5%) of patients on anti-psychotics had red cell folate below 360 nmol/l while all our controls had red cell folate within normal reference range of 360-1460 nmol/.[11,21] Mean red cell folate levels for anti-psychotic naïve, patients on anti-psychotic and controls were 350.23 ± 0.54 nmol/l, 370 ± 0.70 nmol/l and 370 ± 0.51 nmol/l respectively. Compared with control, the p-values for anti-psychotic naïve patients and patients on anti-psychotic were 0.0001 and 0.3500 respectively, Tables 3 and 4.

Serum cobalamin: serum cobalamin levels were evenly distributed and within the normal reference range of 20–680 pmol/[11] in all patients and controls with p-values 0.9122 and 0.6221 for anti-psychotic naïve and patients on ant-psychotic when compared with controls, Tables 3 and 4.

Comparison of the variables amongst the three groups of subjects: When these variables were compared amongst the three groups of subjects, serum cobalamin and red cell folate had p-values of 0.641 and 0.002 respectively, Table 5.

Logistic regression model of predictors of serum cobalamin and red cell folates levels: This was built using the parameters of haemogram as predictors of serum cobalamin and red cell folate status. Reticulocyte count had about 8 times the likelihood of influencing low red cell folate, though not statistically significant (odds ratio = 8.18; 95% CI = 0.010 > 1.000; p = 0.156) and about 3 times the likelihood of influencing low serum cobalamin, also not statistically significant (odds ratio = 2.90, 95% CI = 0.025 > 1.000, p = 0.094), Table 6. None of the other factors in haemogram had statististically significant influence on neither low red cell folate nor low serum cobalamin levels, Table 6.

4. Discussion

A total of 198 subjects were recruited into this study. This comprises of 66 each of anti-psychotic treatment, treatment naïve psychiatric patients and healthy blood donors as controls. Schizophrenia was the commonest diagnosis among all patients; hence significant findings were made in this group of subjects.

From this study, the prevalence of red cell folate deficiency was 13.6% and 4.5% in anti-psychotic naïve psychiatric patients and psychiatric patients on anti-psychotics. This value is high compared with 3% seen among the general population. Five out of nine anti-psychotic naïve psychiatric patients with low red cell folates were also found to have neutrophil hypersegmentation and macrocytosis, highly suggestive of megaloblastic anaemia.

Table 3
Comparing serum cobalamin and red cell folate of anti-psychotic naïve subjects with that of the control.

Variables	Subjects Mean ± SD	Control Mean ± SD	T Statistics	df	p-value
Cobalamin (pmol/L)	160.79 ± 1.17 140.2–180.5	160.77 ± 0.89 141.3–179.1	0.1105	130	0.9122
Folate (nmol/L)	350.23 ± 0.54 350.5–380	370.07 ± 0.51 357.1–378.5	217	130	0.0001

Table 4
Comparing serum cobalamin and red cell folate of subjects on anti-psychotic with that of the control.

Variables	Subjects Mean ± SD	Control Mean ± SD	T Statistics	df	p-value
Cobalamin (pmol/L)	160.84 ± 0.73 140.2–180.5	160.77 ± 0.89 141.3–179.1	0.4940	130	0.6221
Folate (nmol/L)	370.17 ± 0.70 350.5–380	370.07 ± 0.51 357.1–378.5	0.938	130	0.35

Table 5
Comparison of mean values of parameters in the 3 groups (Analysis of Variance – ANOVA).

Parameters	Study groups	n	Mean ± SD	df	F-test	P-value
Cobalamin (pmol/l)	Control	66	16.77 ± 0.89			
	Naive	66	16.79 ± 1.17	2	0.45	0.641
	Treatment	66	16.84 ± 0.73			
Folate (nmol/l)	Control	66	37.07 ± 0.51			
	Naïve	66	35.23 ± 0.54	2	6.71	0.002
	Treatment	66	37.17 ± 0.70			

Table 6
Logistic regression of factors predicting Serum Cobalamin and red cell folate levels.

Haemogram	Variables	P-value	Odds ratio	95% confidence interval
MCH	Serum cobalamin	0.538	1.31	0.559–3.046
	Red cell folate	0.464	1.20	0.738–1.945
MCHC	Serum cobalamin	0.351	1.48	0.653–3.315
	Red cell folate	0.226	1.28	0.860–1.900
MCV	Serum cobalamin	0.284	0.84	0.604–1.158
	Red cell folate	0.784	1.02	0.883–1.179
PCV	Serum cobalamin	0.247	1.52	0.749–3.081
	Red cell folate	0.057	1.47	0.989–2.193
Platelet count	Serum cobalamin	0.318	0.96	0.922–1.996
	Red cell folate	0.301	0.97	0.955–1.987
Reticulocyte count	Serum cobalamin	0.094	2.90	0.025–1.000
	Red cell folate	0.156	8.18	0.010–1.000
Reticulocyte index	Serum cobalamin	0.099	0.01	0.000–57.124
	Red cell folate	0.154	0.01	0.000–98.356
WBC	Serum cobalamin	0.287	0.68	0.337–1.379
	Red cell folate	0.186	0.82	0.612–1.100

Mean red cell folate has been reported to be lower among psychiatric patients on admission compared to those on out-patients follow-up.[21] However, in this study significantly low mean red cell folate was found amongst anti-psychotic naïve psychiatric patients compared with the controls, p-value 0.0001 but not with psychiatric patients on anti-psychotics, p-value 0.3500 despite the fact that the two groups of respondents were recruited from out-patient clinic. This finding is similar to that of other researchers[22,23] while other researchers[15,16,24] reported normal values.

No significant difference was noticed in the mean red cell folate level of psychiatric patients on anti-psychotics and the controls in this study. This may be due to the fact that anti-psychotic has significant effect on normalization of red cell folate of patients with psychiatric disorders.

None of the subjects had serum cobalamin level lower than the normal reference range (20–680 pmol/l). That is serum cobalamin was found to be normal in all subjects and controls. The report is similar to that of other authors[6] but differ from others.[16,22,24] Significantly high mean serum cobalamin compared with controls found by some authors was mainly among patients with Schizophrenia,[16] although in this same group of patients low values was also reported by other authors.[22,24] The finding of normal serum cobalamin levels in all subjects in this study could be explained based on the fact that most of them are recruited from out-patients clinic where they are apparently stable compared with those on admission in the wards or emergency units. Also, the fact that most except two of the subjects are less than 12 months on medication ruled out the influence of anti-psychotics on serum cobalamin. The differences in our findings from that of other researchers could be also due to serum cobalamin assay methods. In this study, ELISA method which is said to be more sensitive but less specific was used, while most other researchers used Cobas E4.

Of all the factors in the haemogram, reticulocyte count had about 8 times and about 3 times the likelihood of influencing low red cell folate and low serum cobalamin respectively, though not statistically significant in both. None of the other factors had influence on low red cell folate or serum cobalamin.

An obvious pattern in the results of the three groups of respondents in this study was the closeness of the folate values to the lower limit of 360 nmol/L. This suggests that the red cell folate reference interval for this environment may be different from the Caucasian value.[11,21]

5. Conclusion

All our patients had Serum cobalamin levels within the reference interval, the same can be said of the RBC folate levels of the greater percentage (95.5%) of psychiatric patients on psychotropic drugs. However, folate deficiency was present in 13.6% of newly diagnosed anti-psychotic naive psychiatric patients with few of them having neutrophil hypersegmentation (7.6%) and macrocytosis (4.5%).

RECOMMENDATIONS: Since there was a significantly low mean red cell folate levels of antipsychotic-naïve patients compared with that of controls, routine folic acid supplement should be part of the initial treatment with antipsychotic drugs in these patients. More studies need to be carried out on a larger number of respondents since there is need to have reference values for our environment in view of the low red cell folate recorded in most of our subjects.

Conflict of interest

The authors declared that there is no conflict of interest.

References

1. Kastrup MC, Ramos A. Global mental health. *Dan Med Bull*. 2007;54:42–43.
2. Srivastava K. Urbanization and mental health. *Ind Psychiat J*. 2009;18:75–76.
3. Hoffbrand AV, Green R. Megaloblastic anaemia. In: Hoffbrand AV, Catovsky D, Tuddenham EGD, eds. *Postgraduate haematology*. 5th ed. Massachusetts: Blackwell Publishing; 2005:60–83.
4. Fallon LF, Wienclaw RA. Nutrition and mental health. *Gale encyclopaedia of mental health*. 2009;2:785–791.
5. Hoffbrand AV, Moss PAH, Pettit JE. *Megaloblastic anaemias and other macrocytic anaemias in Essential Haematology*. 6th ed. West Sussex: Blackwell Publishing; 2010. 50–64.
6. Lerner V, Kanevesky M, Dwolatzky T, Rouach T, Kamin R, Miodownik C. Vitamin B12 and Folate serum levels in newly admitted psychiatric patients. *Clin Nutr*. 2006;25:60–67.
7. Arroll MA, Wilder L, Neil J. Nutritional intervention for the adjunctive treatment of Schizophrenia: a brief review. *Nutr J*. 2014;13:91.
8. Suliman ME, Lindohlm B, Barany P, Berqstrom J. Hyperhomocysteinaemia in chronic renal failure patients: relation to nutritional status and cardiovascular disease. *Clin Chem Lab Med*. 2001;39:734–738.
9. Kamat PK, Mallonee CJ, George AK, Tyaqi SC, Tyaqi N. Homocysteine, alcoholism and its potential epigenetic mechanism. *Alcohol Cline Exp Res*. 2016;40:2474–2481.
10. Haj Mouhamed D, Ezzaher A, Neffati F, Douki W, Najjar MF. Effect of cigarette smoking on plasma homocysteine concentration. *Clin Chem Lab Med*. 2011;49:479–483.
11. Carmel R, Green R, Rosenblatt DS, Watkins D. Update of cobalamin, folate, and homocysteine. *Am Soc Hematol Educ Program*. 2003;62–81.
12. Mokaka MT, Rataemane ST, Santos M. Disability claims on psychiatric grounds in the South African context: a review. *S Afr J Psych*. 2012;18:34–41.
13. Aaron S, Kumar S, Vijayan J, Jacob J, Alexander M, Gnanamuthu C. Clinical and laboratory features and response to treatment in patients presenting with vitamin B12 deficiency-related neurological syndromes. *Neurol India*. 2005;53:55–58.
14. Alturabi EK, Bolad AK, Tambal A, Hamad A, Ali M. Vitamin B12 and folate status in sudanese psychiatric patients. *As J Biomed Pharm Sc*. 2013;3:22–26.
15. Saedisomeolia A, Djalali M, Moghadam AM, Ramezankhani O, Najmi L. Folate and Vitamin B12 status in schizophrenic patients. *J Research Med Sci*. 2011;16:437–441.
16. Bazuaye GN. Prevalaence of Vitamin B12 (Cobalamin) deficiency in Psychiatric patients in Benin City. A dissertation submitted to National Postgraduate Medical College of Nigeria; 2002.
17. 2006 population census. Nigeria National Population Commission. <www.population.gov.ng>.
18. Goldberg DP, Williams P. *A user's guide to the general health questionnaire*. Windsor: NFER-Nelson; 1988. 18–24.
19. World Health Organization. The ICD – 10 classifications of mental and behavioral disorders. Clinical descriptions and diagnostic guidelines. Geneva: WHO; 1992. p. 24–41.
20. Araoye MO. Subject selection in Research methodology with statistics for health and social sciences. 1st ed. Nathadex publishers Ilorin; 2003. p. 115–29.
21. Carney MW, Chary TK, Laundy M, et al.. Red cell folate concentrations in psychiatric patients. *J Affect Disord*. 1999;19:207–213.
22. Ebesunun MO, Eruvulobi HU, Olagunju T, Owoeye OA. Elevated plasma homocysteine in association with decreased vitamin B12, folate, serotonin, lipids and lipoproteins in depressed patients. *Afr J Psychiat*. 2012;15:25–29.
23. García-Miss MDR, Perez-Mutul J, Lopez-Canul B, et al.. Folate, homocysteine, interleukin-6, and tumor necrosis factor alfa levels, but not the methylenetetrahydrofolate reductase C677T polymorphism, are risk factors for schizophrenia. *J Psych Res*. 2010;44:441–446.
24. Lindenbau J, Healton EB, Savage DG. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med*. 1988;318:1720–1728.