Folate Deficiency: A Major Cause of Anaemia in Chronic Lymphocytic Leukemia among Patients in Makudi, Nigeria

Okoli Robertson Onyeka[1]*, Okolie Ifechukwude[1], Myke-Mbata Blessing Kenechi[2], Mke Aondover[1], and Alao Olusayo Olayinka[1]

[1]*Department of Haematology, Benue State University, Makurdi, Nigeria and* [2]*Department of Chemical Pathology, Benue State University, Makurdi, Nigeria*

***Corresponding author**: Dr. Myke-Mbata Blessing Kenechi. Email address:kcbless2001@gmail.com

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Abstract

BACKGROUND

Folate supplementation is often abhorred in malignancy, following fear of worsening disease as a result of folate therapy which is believed to increase the proliferation of malignant cells. However, few studies have re-evaluated the stance on the exclusion of folate in the management of some malignancies. Therefore, this study evaluated the concentration of folate in Chronic Lymphocytic Leukemia(CLL) patients to improve management outcomes.

MATERIALS AND METHODS

This was a cross-sectional study carried out among CLL patients seeking care in Benue State University Teaching Hospital. Thirty-six CLL patients and 36 healthy age and sex-matched controls were recruited for the study. Full blood count, peripheral blood film, Erythrocyte sedimentation rate, and serum folate levels were determined. Folate deficiency was defined as a concentration of <3.0ng/ml and a haemoglobin concentration of <11g/dl was described as anaemia. Data obtained from the study were analysed using Statistical Package for Social Sciences (SPSS) version 20.0.

RESULTS

Folate deficiency was more common in the CLL group (27.8%) compared to controls (2.8%), P <0.05. There was a lower mean haemoglobin, platelet count and neutrophil count in the folate-deficient CLL patients compared to the non-deficient patients (p < 0.05). The mean corpuscular volume was not significantly different; however, a high red cell distribution width was noted in the CLL group which was more pronounced in the folate-deficient CLL subgroup.

CONCLUSION AND RECOMMENDATIONS

There was a high prevalence of folate deficiency among patients with CLL in Makurdi, Benue state. This deficiency was more pronounced among CLL patients with anaemia and high-risk disease. Anaemia in CLL patients should be fully investigated and the possibility of micronutrient supplementation considered especially in patients with high-risk disease.

Keywords*: Chronic Lymphocytic Leukaemia, Folate, Anaemia, Quality of Life*

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Introduction

Folates are vital micronutrients often required for normal metabolism, cellular regeneration and proliferation, and their deficiencies can result in cytopaenias which inadvertently leads to anaemia, reduced immunity and poor heamostasis[1]. In most developing countries, there is a high prevalence of folic acid deficiency due to poor socio-economic status and poor food preparation methods which is worsened by lack of policies on folic acid fortification of processed food[2,3]. Therefore, increased utilization of folic acid in diseases such as malignancies, in such environments is most likely to lead to severe folate deficiency.

Chronic Lymphocytic Leukemia(CLL) is a clonal malignancy of B-lymphocyte in which there is proliferation and accumulation of mature-looking lymphocytes in the blood, bone marrow and other lymphoid organs[4]. This disease is often complicated by cytopaenias such as anaemia, thrombocytopaenia and neutropenia which increase morbidity in CLL[4]. Folate deficiency in CLL patients can worsen the cytopenia and inadvertently reduce the quality of life[6].

Anaemia is an important feature in patients with CLL as it is critical for the staging of the disease. A study carried out by Salawu *Et al.*,[5] in southwest Nigeria observed that 74.7 % of CLL patients had anaemia at presentation. The common causes of anaemia in CLL include bone marrow infiltration, autoimmune haemolytic anaemia and micronutrient deficiencies6. Nutritional anaemia is common in CLL due to a deficiency of iron, folic acid/vitamin B12 or a combined deficiency 6. It has also been observed that patients with lymphoid malignancies also have an increased incidence of malabsorption of nutrients, especially folic acid.[1] Therefore, this study evaluated the serum folate concentration of CLL participants in Benue State University Teaching (BSUTH) Makurdi in comparison with age-matched non-CLL patients.

Materials and Methods

Study design

This was a cross-sectional study carried out at Benue State University Teaching (BSUTH), from October 2018 to September 2019. The Participants were recruited from chronic lymphocytic leukaemia patients attending the Haematology Out-Patient Clinic, and the control group comprised apparently healthy members of staff and blood donors at BSUTH. Consenting CLL patients with a diagnosis of CLL supported by blood film and full blood count (FBC) with or without bone marrow aspirate cytology/biopsy and healthy consenting members of staff and blood donors at BSUTH, whose blood films were not suggestive of CLL served as control. On the other hand, CLL patients on management for megaloblastic anaemia with folic acid replacement; history of blood transfusion that is less than 4 months; Patients on multivitamin supplementation as well as Control participants on multivitamin supplementation; non-consenting as well as non-optimal haemogram were excluded from the study. Folate deficiency was defined as a concentration of <3.0ng/ml and a haemoglobin concentration of <11g/dl was described as anaemia.

Sampling and data collection

Purposive sampling was used to recruit 36 consecutive consenting CLL patients (12 males;24 females) and thirty-six age and sex-matched controls (18 males and 18 females) and this was done in the same period. The sample size for participants was determined by using a formula for studies that estimate a quantity of interest with a specified precision.

Thus:

$$n = \frac{\pi(1-\pi)}{e2}$$

Where: n: Sample size

π: Estimated proportion of folate-deficient CLL patients

e: required size of standard error (5%)

The estimated proportion of lymphoid malignancy (including CLL) patients with folate deficiency was less than 10%, hence:

$$n = \frac{0.1\ (1-0.1)}{0.052} = 36$$

Thus, the minimum sample size for this study was 36 participants. Socio-demographic data was obtained using a self-developed questionnaire and interviewer-administered.

Sample collection

The study involved the collection of 7ml of venous blood from each participant. The sample was then divided into two portions, one placed into potassium EDTA and plain non-

coagulant-containing vacutainers. The sample in EDTA was used for full blood count (FBC), peripheral blood film and reticulocyte count which enabled the categorization of the patients in accordance to RAI staging of CLL. The blood in plain vacutainer was allowed to stand for a minimum of two hours to clot and retract. The retracted sample was centrifuged at 2000g for 20 minutes after which serum was decanted and stored at -20^{0}C till the time for folate analysis. Serum folate assay was done through Enzyme-linked Immunosorbent Assay (ELISA) method using a Monobind test reagent kit by Monobind Inc. Lake Forest, CA52630 USA.

Data analysis

Data were analysed by SPSS version 20. The continuous variables were expressed as mean ± standard deviation. A paired samples t-test was used to compare the mean of haematological indices and serum folate of the CLL and Control group, while the Chi-square test and Wilcoxon signed rank were used for ordinal and skewed data. The statistically significant level was set at $p < 0.05$.

Ethical considerations

Ethical approval was obtained from the Ethics Review Board of Benue State University Teaching Hospital (BSUTH) Makurdi, Nigeria (BSUTH/MKD/HREC/2013B/2018/0C06). Consent was sought and obtained in writing from each participant and participants' right of confidentiality protected.

Results

Socio-demographic characteristics of participants

Seventy-two (72) participants who met the inclusion criteria for this study were recruited. A total of 36 CLL participants and 36 control participants were recruited. The age, sex and occupational distribution of the participants are also shown in Table 1. About 58% of the CLL participants were farmers while 19.4% were civil servants, 11.1% were traders and 11.1% were of other occupations.

Rai staging of CLL participants

The distribution of Rai staging of the CLL participants is shown in Table 2. About 77.8% of the CLL participants had high-risk (stage III and IV) disease while 22.2% had intermediate-risk (stage I and II) disease none had low-risk (stage 0) disease. The haematological indices of CLL participants and control participants are shown in Table 3. The concentration of total white cell count of CLL participants was significantly($p \leq 0.05$) higher than control (66.48 ± 66.35 x109 /L; 5.6 ± 1.3 x 109/L; p= 0.000). Additionally, haemoglobin concentration of CLL participants was significantly($p \leq 0.05$) less than the control (9.14 ± 2.02 g/dl; 13.8 ± 1.7 g/dl; p= 0.000).

Table 1:
Age, Sex and Occupational Distribution of the Study Participants

Variables		CLL frequency n(%)	Control frequency n(%)
Age group(years)	41-50	5(13.9)	12(33.3)
	51-60	13(36.1)	13(36.1)
	61-70	12(33.3)	8(22.2)
	≥71	6(16.7)	3(8.3)
	Total	100	100
Sex	Male	12(33.3)	18(50)
	Female	24(66.7)	18(50)
	Male: Female Ratio	1:2	1:1
Occupational	Farming	21 (58.3)	14 (39.8)
	Civil servant	7 (19.4)	18 (50)
	Trading	4 (11.1)	3 (8.3)
	Others	4 (11.1)	1 (2.8)
	Total	36 (100)	36 (100)

The serum folate for CLL participants was significantly ($p \leq 0.05$) lower than the control (5.88 ± 3.78 ng/ml;7.8 ± 4ng/ml;p= 0.042). The platelet count of CLL participants was significantly less ($p \leq 0.05$) than that of the control group (135 ± 85 x 109/L;194 ± 64 x 109/L; p = 0.002). Other haematological indices of the CLL and the control groups are shown in Table 3.

The prevalence of folate deficiency in the control group was 2.8% which was significantly lower than the 27.8% observed in the CLL group with $\chi 2 = 8.69$ and $p = 0.003$ as shown in table 4. Table 5 shows a comparison of haemoglobin concentration, mean corpuscular volume and red cell distribution width between folate-deficient and folate-non-deficient CLL participants. The mean ± SD of haemoglobin concentration in folate-deficient CLL participants was significantly lower than that of the non-deficient CLL participants, 7.9 ± 1.9g/dl and 9.6 ± 1.9 g/dl respectively with p = 0.020.

The Spearman correlation coefficient of serum folate, haemoglobin concentration, platelet count and neutrophil count is shown in Table 6. The Rai stage had a significant ($p \leq 0.05$) negative correlation with serum folate. Haemoglobin concentration, neutrophil count and platelet count had no significant ($p \leq 0.05$) positive correlation to serum folate.

Table 2:
Rai Staging of CLL Participants

Stage (risk category)	Frequency (n)	Frequency (%)
I (intermediate risk)	6	16.7
II (intermediate risk)	2	5.6
III (high risk)	15	41.7
IV (high risk)	13	36.1
Total	36	100

Table 3:
Blood Parameters of Control and CLL Participants

Variable	Control (mean ± SD)	CLL (mean ± SD)	p-value
T WBC(x 109 /L)	5.63 ± 1.26	66.48 ± 66.35	0.000*
Neutrophil count(x 109 /L)	2.51 ± .98	6.23 ± 4.84	0.001*
Lymphocyte count(x 109 /L)	2.75 ± .71	58.18 ± 59.64	0.000*
MXD(x 109 /L)	.37 ± .37	1.09 ± 1.20	0.003*
HB concentration (g/dl)	13.81 ± 1.70	9.14 ± 2.02	0.000*
Haematocrit (%)	41.75 ± 5.02	37.80 ± 5.02	0.000*
Mean cell volume(fl)	83.94 ± 8.25	85.33 ± 7.81	0.364
MCH(pg)	27.28 ± 3.10	29.15 ± 3.80	0.027*
MCHC (g/dl)	31.96 ± 1.75	30.85 ± 2.90	0.468
RDW SD(fl)	46.87 ± 4.13	53.41 ± 6.12	0.000*
Platelet count(x 109 /L)	194 ± 64	135 ± 85	0.002*
SERUM FOLATE (ng/ml)	7.81 ± 4.02	5.88 ± 3.78	0.042*

*significant at $p < 0.05$

Table 4:
Frequency of Folate Deficiency in the Control and CLL Group

Serum folate (ng/ml)	Control n (%)	CLL n (%)	χ2	P
Non-deficient (≥3)	35(97.2)	26(72.2)	8.69	0.003*
Deficient <3	1 (2.8)	10 (27.8)		
Total	36 (100)	36 (100)		

The mean folate concentration is lower in CLL participants with stage III and stage IV disease compared to those with Ria stage I and II.

Discussion

Serum folate is an indicator of the quantity of folate available for tissue metabolism. The study showed a high prevalence of folate deficiency in CLL patients compared to controls which was statistically significant.

This is similar to the observation made by Kim *Et al.*,[7] in a study that assessed the serum folate level of patients with lymphoid malignancy. Also, Epstein-Peterson *Et al.*,[8] observed a lower mean serum folate in patients with malignancies compared to control participants in a study that assessed the serum folate level in hospitalized patients with malignancies.

Table 5:
Comparison of the Mean of Haemoglobin Concentration, Mean Corpuscular Volume, and Red Cell Distribution Width between Folate-Deficient and Folate-non-Deficient CLL Participants

Variable	Serum folate Deficient	Serum Non-deficient	p-value
Haemoglobin concentration(g/dl)	7.9 ± 1.9	9.6 ± 1.9	0.020*
Mean corpuscular volume (fl)	83.9 ± 7.2	85.8 ± 8.1	0.529
Red cell distribution width SD	56.2 ± 7.1	52.3 ± 5.4	0.083

Table 6:
Correlation between Serum Folate and Rai Stage, Neutrophil Count, Platelet Count and Haemoglobin Concentration in CLL Participants

Variable	Spearman's correlation	p-value
Serum folate /Rai stage	-0.333	0.048*
Serum folate /Neutrophil count	0.289	0.087
Serum folate /haemoglobin concentration	0.072	0.678
Serum folate/Platelet count	0.308	0.067

Relationship between the different stages of CLL and serum folate.

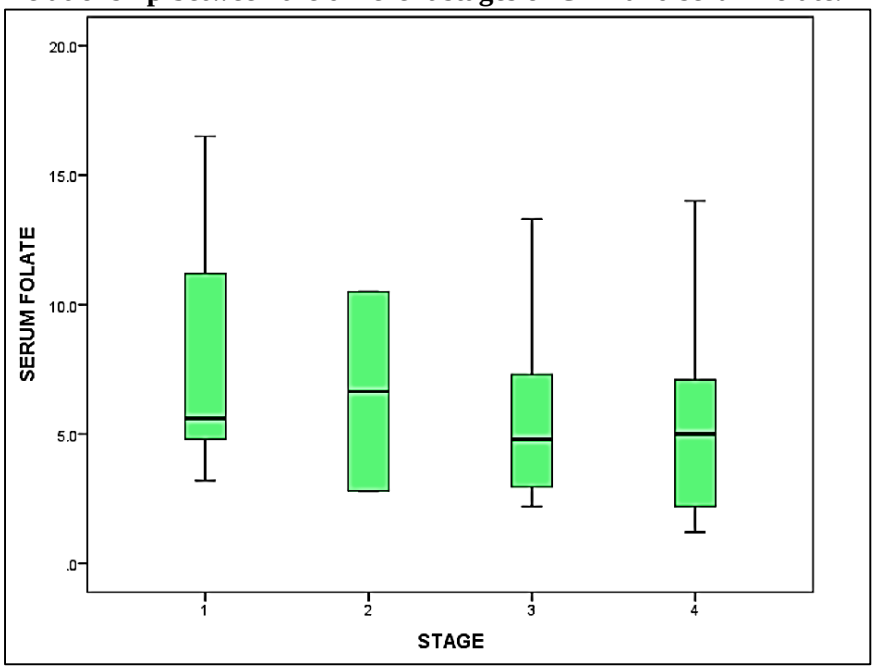

Figure 1:
A box plot showing the relationship between the different stages of CLL and serum folate.

These authors also noticed an improvement in serum folate in patients in remission. The low serum folate could be secondary to increased utilization due to rapid cell turnover seen in lymphoid malignancies poor absorption or reduced dietary intake. However, folate deficiency is a risk factor for some malignancies[7,9], in such instances it will predate the malignancy, which may be the case in some CLL participants. Another factor that could be responsible for the reduced serum folate observed in the study was the age of the participants, folate deficiency is known to be commoner among the elderly[1]. Hence, in this study, the majority of the CLL participants were above 50 years old (>86%).

The study showed a high prevalence of anaemia among CLL participants (75%) which is similar to the finding of Salawu *Et al.*,[5] in Southwest Nigeria and Madu *Et al.*,[10] in Southern Nigeria who observed that 74.4% and 78% respectively of CLL patients had anaemia at presentation. A significant proportion of the anaemic CLL participants in this study also had folate deficiency (33.3%). Many factors are responsible for anaemia in patients with lymphoid malignancy including bone marrow infiltration by malignant cells, anaemia of chronic disease, iron deficiency, autoimmune haemolysis, hypersplenism, nutritional deficiency, malabsorption [6,11]. Most patients will have a combination of two or more of these factors. A significant cause of anaemia in CLL may be due to folate deficiency as this research showed that folate-deficient CLL participants had lower concentrations of haemoglobin. Hence, folate supplementation may improve the haemoglobin concentration of CLL patients, thereby improving the outcome of disease management.

Folate deficiency commonly manifests as macrocytic anaemia, however, in this study the prevalence of macrocytosis (2.8 %) was observed to be the same in the control and CLL groups despite the high prevalence of folate deficiency in the CLL group. The mean corpuscular volume of both groups was not significantly different and was within the reference interval. However, a statistically significant difference was seen in the mean ± SD of red cell distribution width which was higher in the CLL group compared to the control group. This elevated red cell distribution width in CLL patients was more pronounced in the folate-deficient (56.2 ± 7.1fl) subgroup compared with those without folate deficiency (52.3 ± 5.4fl). This combination of normal mean cell volume and elevated red cell distribution width is not a common feature of folate deficiency but is often seen in patients with combined folate and iron deficiency or patients with anaemia of chronic disease coexisting with folate deficiency[12].

The mean serum folate was observed to decrease as the disease progressed. As shown in the study, the mean folate concentration is lower in CLL participants with stage III and stage IV disease compared to those with Ria stage I and II. This is similar to the observation by Kuo *Et al.*, in their study on the relationship between serum folate and disease progression in patients with hepatocellular carcinoma. In their study, they found that low blood folate status could be a risk factor for tumour progression[13]. Singh et al, observed in a prospective study that patients, on the pemetrexed-platinum combination, were better able to tolerate treatment and showed less incidence of severe haematological complications when placed on folate and vitamin B12 supplementation[14].

Limitations of the study

Serum folate alone may not be a sufficient indicator of deficiency of the vitamin, other analytes for example, serum homocysteine and urine formiminoglutamic acid excretion, when combined with the above will be more informative. Some of the patients were on

treatment for CLL already, which could have improved their clinical and haematological indices.

Conclusion and Recommendations

There is a high prevalence of folate deficiency among patients with CLL in Makurdi, Benue state. This deficiency is more pronounced among CLL patients with anaemia and advanced disease. Therefore, screening of CLL patients and possible replacement of folate may improve the outcome of disease management. Anaemia in CLL patients should be fully investigated and the possibility of micronutrient supplementation considered especially in patients with high-risk disease.

Conflict of interest. None declared
Source of funding.

Abbreviations

EDTA - Ethylenediaminetetraacetic acid
ESR - Erythrocyte sedimentation rate
FBC - Full Blood Count
HOPC - Haematology Outpatient Clinic
MCH - Mean corpuscular haemoglobin
MCHC - Mean corpuscular haemoglobin concentration
MCV - Mean corpuscular volume
MXD - Mixed differential
RDW - Red cell distribution width
RDW - CV Red cell distribution width coefficient of variation
RDW - SD Red cell distribution width standard deviation
TWBC - Total white cell count
WBC - White cell count

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