Reference values for Serum levels of Folic acid and Vitamin B12 in a young adult Ugandan population

*Galukande M[1], Jombwe J[2], Fualal J[2], R Baingana[3], Gakwaya A[2]

1. Department of Surgery, School of Medicine, College of Health Sciences, Makerere University
2. Department of Surgery, Mulago National Referral Hospital, Kampala
3. Department of Biochemistry, Faculty of Science, Makerere University

Abstract

Introduction: Reference values may vary significantly among populations and cultures for which dietary intakes may be different. Each laboratory is expected to investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges. This study sought to establish Folic acid and Vitamin B_{12} reference values for young Ugandan adults, which reference values could be used as baseline information for future research, especially related to their role in breast cancer prevention.

Methods: A cross sectional study in which 200 undergraduate student volunteers were enrolled. The minimum sample size was 126 using the Open Epi version 2. Relevant demographics and physical examination parameters were taken as well as blood samples for laboratory tests. Prior ethical approval was granted.

Results: The levels of folic acid were 4.17 – 20ng/ml and remained constant over the age cohorts. The vitamin B_{12} range was 117-1158pg/ml. Both ranges were comparable to ranges seen in other populations.

Conclusion: In as much as the dietary intake for this study population is presumed different from populations in the western countries, the ranges for these two vitamins were similar.

Key words: Reference Serum levels, Folic acid, Vitamin B12

Introduction

The reference values that we find in textbooks and standard laboratory manuals may vary significantly among populations and cultures for which dietary intakes may be different[1, 2, 3]. Each laboratory is expected to investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Reference values for various blood parameters are often obtained through sampling young individuals with a high probability of good health, or a mix of both healthy and non-healthy individuals recruited in hospitals or other health facilities[4].

Folic acid and Vitamin B_{12} are water-soluble vitamins important for nerve, brain function and haemopoesis; they are coenzymes for the synthesis of serotonin and catecholamine neurotransmitters[5, 6, 7].

Folic acid has lately been linked to protection against Cancer of the colon and Cancer of the breast[8-12]. Its protective effect on neurotubal defects in utero is well known[13]. Studies suggest that Vitamin B_{12} too is neurotube defect protective [14].

The impact of multiple potential confounders[15-17] on serum vitamin levels namely age, sex, alcohol, consumption, smoking, BMI (Body Mass Index), Hb (Haemoglobin) MCV (Mean Capsular Volume) ought to be examined as potential covariates in such studies.

This study sought to establish Vitamin B_{12} and Folic acid reference values for young Ugandan adults, which reference values could be used as baseline information for future research, especially related to protective role of folate against breast and colon cancer.

Methods

A Cross-sectional descriptive study carried out among university undergraduate students.
Two hundred Makerere University College of Health Sciences students out of a population of about 700 responded to a call to volunteer, after the objectives

*Correspondence author
Dr. Moses Galukande
Department of Surgery
College of Health Sciences
Makerere University
Email: mosesg@img.co.ug

of the study and the processes involved were communicated to them.

The minimum sample size was 126 (95% confidence interval and using Open Epi Version 2[18] used as appropriate for a descriptive one group cross sectional study), the lowest proportion of folate and vitamin B deficiency in a population taken to be 9% (5-15%)[19].

A pre-tested questionnaire was used for data collection. It included drug history, past illnesses, current health status, smoking and alcohol intake and physical examination including; blood pressure, height and weight. Weight and height (without shoes and with light clothing) were determined using an analogue weighing scale (range: 0.1 – 120kg) and a stationary tape measure for height. The data allowed for the calculation of each subject's body mass index (in Kg m^{-2})

Individuals on treatment for Vitamin B$_{12}$ or folate deficiency or on drugs known to interfere with Vitamin B$_{12}$ or folate uptake such as anticonvulsants, peptic ulcer drugs, trimethoprim and hydrazides were asked not to participate.

All participants consented to the study. Permission was obtained from Makerere University, Faculty of Medicine Ethics and Research committee.

All samples were drawn over a two-day period during the day between 9am and 4pm.

Owing to this range of blood sampling times participants were not fasting.

About 3 mLof blood was drawn into plain vacutainer tubes using standard procedures and serum was separated by centrifugation.

Elecys 2010 analyzer was used in the laboratory for assay for folate and B$_{12}$. For sample processing tube and kit manufacturing instructions were followed. Specific performance data provided by the manufacturer indicates that precision data (reproducibility) and analytical specificity were within acceptable limits.

Data analysis was done using SPSS 11.5 software, descriptive analyses and Pearson correlation coefficient methods were used.

Results

Of the 200 students enrolled for the study, 160 samples were successfully assayed for the folate and 133 samples assayed for Vitamin B12. We excluded 40 samples for folate and 67 for Vitamin B$_{12}$ due to either poor quality of samples or rejection by machine or used for calibration purposes. The values obtained from this study ranged from 4.17 to 20ng/ml for folate levels and 117-1158pg/ml for Vitamin B$_{12}$. When adjusted to the 2.5th to 97.5th percentile they read 4.36 to 17.68ng/ml and 152 to 1029pg/ml respectively. There was no borderline deficiency or deficiency for either vitamin.

Table 1: BMI and laboratory findings of the study population at Makerere University, 2009

	Mean	SD	Range	N
Body Mass Index Kg/M^{-2}	23.1	20.2	22.3 (21.0 - 24.5)	200
Blood Hb g/dl	14.1	4.01	14.4 (12.9 -15.7)	185
Mean cell Volume, MCV (fl)	82.9	50.8	83.5 (79 - 88)	186
Serum folate (ng/ml)	8.34	12.1	7.35 (5.85 - 10.0)	160
Serum Vitamin B$_{12}$ (pg/ml)	469	207	431 (321 - 575)	133

Pearson's correlation coefficient method was used to give information about the degree of correlation as well as the direction of these confounders (age, BMI, Hb and MCV) the degrees of correlation in table 2 lie around zero and 0.25. Indicating a no correlation to a low degree of correlation, implying the tested for cofounders did not significantly influence the ranges we see in this study. Alcohol and cigarette smoking are not shown in the tables, because none of the study participants admitted or indicated that they engage in either smoking or take alcohol on a significantly regular basis.

Table 2: Pearson's Correlations of Vitamins with demographics, BMI, Hb, MCV

	Folate	B$_{12}$
Age	0.130	0.256**
BMI	0.104	0.106
Hb	0.250	-0.023
MCV	0.007	-0.140

** Correlation is significant at the 0.01 level (2 tailed)

As Table 2 shows, age was significantly correlated with vitamin B$_{12}$ (r=0.256, p=0.01). Body mass index, Hb and MCV were not correlated with either folate

or vitamin B_{12}.

Alcohol and cigarette smoking are not shown in the tables, because none of the study participants indicated that they either smoke or take alcohol on a regular basis.

Discussion

The main objective of this study was to establish reference values for serum folate and B_{12} among young health adults. The population studied was deemed representative of young health adults in the country, the Uganda Bureau of Statistics census data suggests that over 20% of the population falls in the age bracket studied[19].

The absolute figures for many of the hematological parameters studied in the sub-Saharan setting differ from those of populations outside Africa suggesting that it is necessary to develop tables of reference values for biomarkers indices specific for the African population[21, 22, 23]

Folate is a water soluble vitamin. Body stores are relatively small and liable unlike iron therefore temporary reduction of dietary intake of folate can produce short term (1month) folate deficiency. This is relieved by a few meals of folate containing foods. Vegetable and fruit are the usual sources of folate[21]. This implies therefore that when tests shown deficiency it is a reflection of some degree of inconsistence in the nature of the diet the individual takes on a regular basis.

The influence of important potential confounders was examined.

Most Ugandan schools prohibit smoking and alcohol for students, even though these are university students where such rules mostly don't apply, not indulging could be a 'hang over effect from high school' or as result of high health consciousness, are plausible explanations.

These expected values for folic acid in the test kits were as follows:

Normal	3.1 – 17.5ng/ml	(7.0 – 39.7 nmol/L)
Borderline deficient	2.2 – 3.0ng/ml	(5.0 – 6.8 nmol/L)
Deficient	< 2.2ng/ml	(<5.0 nmol/L)
Excessive	> 17.5ng/ml	(>39.7 nmol/L)

Values obtained in Australia (2 sites), Europe and in the USA for comparison purposes were;
Australia site 1 ranged between 5.7 to >20.0 ng/ml (n =194), Australia site 2 ranged between 4.6 - >20.0

ng/ml (n=182), Europe ranged between 3.8 to 16.0 ng/ml (n=292) and USA ranges between 8.4 to 35.2 ng/ml (n=167). All these were 2.5th to 97.5th percentile ranges.

The range for Vitamin B_{12} was 152 – 1029pg/ml (2.5th to 97.5th percentile in this study. The laboratory kit measuring range was 30 to 2,000pg/ml. These findings were comparable to values obtained in Germany and USA whose ranges were between 191 and 663 pg/ml (n=291), between 211 and 946 pg/ml (n=178), as 2.5th to 97.5th percentile ranges respectively.

The study had several limitations. Dietary history was not taken, the study subjects for the most part live in the urban setting, there could be variations in the type of diet available in the rural and urban settings this could limit the generalizability of these findings, we also anticipate better availability of vegetables and fruits in most rural settings.

Specimen collection was not conducted at exactly the same time of day for all subjects. The temperature and length of blood storage have been shown to cause alternations in the values for some hematological parameters, although these differences should have been randomly distributed through out the study population. All blood samples were transported at ambient temperature and were processed within the time limits recommended for these bioassays investigations.

Conclusion

The vitamin B12 and folate levels among this young adult population were comparable to values obtained from Western Europe and the USA. This is contrary to what would be expected given the fact that dietary intakes are expected to be different. A comparative survey of food intake could possibly shed light on these unexpected similarities. The values obtained here can be used as baseline data for further research.

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References

1. Alvaro R, Eduardo DS, Paolo B, Hugo DP, Maria M, Félix L. Vegetables, Fruits, and Related Nutrients and Risk of Breast Cancer: A Case-

Control Study in Uruguay. *Nutrition and Cancer.* 1999; 35 (2): 111-119

2. Bain B J. Ethnic and sex differences in the total and differential white cell count and platelet count. *J.Clin. Pathol.* 1996;49;664-666.

3. Dugdale A E. Predicting iron and folate deficiency anaemia from standard blood testing: The mechanism and implications for clinical medicine and public health in developing countries. *Theoretical Biology and Medical Modeling* 2006; 3:34

4. Wahlin A, BackmanL, Hultdin J, Adolfsson R and Nilsson LG. Reference values for serum levels of Vitamin B_{12} and folic acid in a population-based sample of adults between 35 and 80 years of age. *Public Health Nutrition* 2002; 5(3): 505-511

5. Carrey MWP, Toone BK, Reynolds EH. S-adenosylmethionine and effective disorder. *Am J. Med* 1987; 83 (suppl.5A): 104-6

6. Levitt AJ, Joffe RT. Folate Vitamin B_{12} and life course of depressive illness. *Bio Psychiatr.* 1989; 25: 867-72

7. Berry RJ, Carter HK, Yang Q. Cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr.* 2007; 86:265-267.

8. Tamini RM, Lagion P, Adami HO, Trichopoulos prospects for chemoprevention of cancer. *Journal of Internal Medicine.* 2002; 251:286-300

9. Figueiredo JC, Levine AN, Grau MV et al. Colorectal Adenomas in a randomized folate trial: The role of Baseline Dietary and Circulating folate levels. *Cancer Epid Biomarkers & Prevention.* 2008; 17: 2625-2632.

10. Maruti SS, Ulrich CM, White E. Folate and one–carbon metabolism nutrients from supplements and diet in relation to breast cancer risk. *American Journal of Clinical Nutrition* 2009; 89(2): 624-633.

11. Slattery L M, Potter D J, Samowitz W, Schaffer D, Leppert M. Methylene tetrahydrofolate reductase, Diet and risk of colon cancer. *Cancer Epidemiology, biomakers and prevention.* June 1999; Vol 8: 513-518

12. Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, Olson JE, Therneau TM, Folsom AR. Dietary Folate intake, Alcohol and risk of Breast cancer in a prospective study of post menopausal women. *Epidemiology.* July 2001; 12(4): 420 -8

13. Sadler TW. Longman's medical embryology 8th edition. 2000; 19:426

14. Molloy Am, Kirke PN, Troedle JF et al. Maternal Vitamin B12 status and risk of neural tube defects in a population with High neural tube defect prevalence and no folic acid fortification. *Paediatrics* 2009; 123(3): 917-923.

15. Waggemans RM, De Groot LCPGM, Haller J. Factors related to plasma Folate and Vitamin B_{12}. The SENECA study. *Int J Food Sci. Nutr.* 1997; 48:141-50

16. Benton D, Haller J, Fordy J. The Vitamin status of young British adults, *Int. J. Vit. Nutr. Res.* 1997:34-40

17. Rosenberg I H. Folate in: Hartz SC, Russell RM, Rosenberg IH. Eds. Nutrition in the elderly. *London: Smith-Gordon* 1992; 135-40. 1.http://www.openepi.com/OE2.3/samplesize/SSPropor.htm

18. Hanger HC, Sainsburg R, Gilchrist NL, Beard MEJ, Duncan JM. A community study of vitamin B12 and folate levels in the elderly. *J Am. Geriatr. Soc* 1991; 39:1155-9

19. UBOS (Uganda Bureau of Standards 2002) www.ubos.org/onlinefiles/uploads/ubos/.../UBOS%20SSPS

20. Lugada E S, Mermin , Kaharuza, Ulvestad E, Were W, Langeland, Asjo B, Malamba S, Dowing R. Population –based heamtologic and immunologic reference values for healthy Ugandan population. *Clin. Diagn. Lab. Immunol* .2004;11;3:29-34

21. Tugume SB, Piwowar E M, Lutalo T, Mugyenyi R M, Grant R M, Mangeni F W, Pattishall K, Katongole-Mbidde E. Haeamotological reference ranges among healthy Ugandans . *Clin. Diagn. Lab. Immunol.*1995,2:233-235

22. Badenhorst CJ, Fourie K, Steyn P, Jooste C, Lombard J, Bourne L, Slazus W. The haematological profile of urban-black Africans aged 15-64 years in the Cape Peninsula. *East Africa Med. J* 1995; 72: 19-24

23. Kratz A, Lewandrowski KB. Normal reference Laboratory Values. *N Eng J Med.* 1998; 339 (15): 1063 - 1072