The prevalence of vitamin B12, folic acid and iron deficiency in healthy relatives of patients admitted at regional referral hospitals in Rwanda: a cross sectional study

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ABSTRACT

Background: The clinical observations at teaching hospitals across Rwanda have anecdotally documented high rates of reversible megaloblastosis due to a deficiency in B complex vitamins. This study aimed to determine the prevalence of vitamin B12, folic acid and iron deficiency in a cross-section of a healthy adult Rwandan population.

Methods: This study enrolled 191 healthy participants from different regions of Rwanda. All study subjects signed informed consent, completed a pre-designed questionnaire covering their general demographics, geographical distribution and dietary habits, and underwent a neurological examination. Complete blood count, serum vitamin B12, folic acid, ferritin and C-reactive protein levels were measured. StataMP 13 (64 bit) was used for statistical analysis. The statistically significant p value was set to < 0.05.

Results: The point-prevalence of vitamin B12 deficiency using the standard WHO definition was 9.9%. Low or low-normal vitamin B12 levels were observed among 39/108 (36%) of subjects between 18-35 years-of-age. Iron deficiency was found in 7/191 (3.6%) of participants. None of the participants in this study were found to have a folic acid deficiency.

Conclusion: The proportion of low vitamin B12 was much higher than that reported in developed countries.

Recommendation: Further research is needed to elucidate the leading causes of vitamin B12 deficiency in Rwanda.

Key words: vitamin B12, B12 deficiency, reticulocytes, micronutrient, nutrition, Rwanda.

INTRODUCTION

Micronutrient deficiency, also known as the "hidden hunger", is a global health threat that it is frequently linked to impaired hematopoiesis which presents as anemia. It is especially prevalent in patients that have iron, vitamin B12 or folic acid deficiency. The combined deficiency of multiple different micronutrients is a common problem in the developing world [1][2][3]. There are several reports across low-income countries that highlight the clinical presentation of a deficiency in B group vitamins presenting as severe forms of anemia [4][5] and also unusual presentations that include abnormalities in the nervous system [6], the skin or its appendages [7].

From the medical textbooks we learn that the etiologies attributed to these deficiencies vary from inadequate nutritional intake [8], malabsorption [9][10], chronic use of high risk medications [11], infections [12] and auto-immune pathology, such as pernicious anemia, notoriously known to lead to a deficiency in vitamin B12 [13]. Triggered by several reports on a possibly significant dietary component, especially in developping countries, several scholars have studied the relationship between the nutritional intake and the measured micronutrient levels. The obtained results are still not conclusive for a nutritional deficit as the primary cause for the micronutrient deficient state in those countries [14].

The clinical observations at the emergency room and medical inpatient service at CHUK (Centre Hospitalier Universitaire de Kigali) have noted symptomatic anemia due to vitamin B12 and folic acid deficiency among patients who were repetitively requiring blood transfusions [15]. During the history taking, those index cases reported a significantly low consumption rate in animal protein and the supplementation of those missing vitamins reversed their cytopenias. These observations have raised concerns regarding a potential endemic deficiency in these vitamins in the population at large.

Reference was made to the existing and growing data on micronutrient deficiency in the pediatric population and their clinical implication in Rwanda, whereby more than 30% of the population under the age of 5 was reported to be stunted [16][17]. There was a crucial need to profile the undernutrition state in the adult population in order to determine its extent and hopefully propose a feasible therapeutic approach if it was found to be a problem of major public health significance.

Aim: This study aimed to determine the prevalence of vitamin B12, folic acid and iron deficiency, and to shed light on dietary habits at a larger scale on a cross-section of a healthy adult Rwandan population.

METHODS

Participants and enrollment

This was a cross-sectional study enrolling a healthy population composed of attendants or visitors of hospitalized patients from 15th April 2016 to May 4th 2016. The study sites included CHUB (Centre Hospitalier Universitaire de Butare) in the Southern Province, Bushenge Provincial Hospital in the Western Province, Nemba and Kinihira Hospitals in the Northern Province, Masaka Hospital in the periphery (new settlement) of the capital city of Kigali and Rwamagana Hospital in the Eastern Province.

The study participants were identified at the time they visited their hospitalized relatives. The local social worker and/or senior nurse facilitated a meeting in which all potential study participants convened to the hospital's conference room for a short educational session on "micronutrient deficiency that lead to anemia." At the same venue we provided all necessary details regarding this study.

The individuals ages 18 years old and above, who were capable of understanding the purpose of the study and signed the informed consent, were recruited into the study. We excluded those with previous diagnosis of vitamin B12, folic acid or iron deficiency, those who had used pure vitamin B12, folic acid or iron supplements and the visitors of patients admitted on malnutrition wards.

The enrollment was consecutive until the desired sample size was reached. The sample size calculation was based on prior reporting on the prevalence of iron deficiency (range between 8% to 15%) at the community level in Rwanda [18][19].

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of the University of Rwanda; College of Medicine and Health Sciences.

Data collection and laboratory measurements

We used a pre-designed data collection form that captured general demographics, dietary habits and site of residence among other parameters of interest.

Diet assessment was performed by documenting the minimum frequencies of consumption of several nutrients, on an estimated recall of one month for example minimum of once daily/ minimum of once weekly/ minimum of once monthly/ very rarely consumed or never. The consumption of animal protein and dairy products was also scored as 0 (very rarely consumed or never), 1 (minimum of once monthly), 2 (minimum of once weekly) and 3 (minimum of once daily) in the attempt to reflect at best the consumption of different nutrients known to be source of the vitamins in question considering personal dietary preferences or nutrients' availability.

The documentation on pertinent past medical history and neurological problems relevant to vitamin B12 deficiency such as paresthesias, memory impairment, depression, irritability and decreased libido were all self-reported and were recorded in a scoring system that attributes 1 point to each question answered affirmatively.

The proprioception and vibration sense were tested by positioning the first metatarso-phalangeal joints of both great toes and placing a vibrating 128 hertz tuning fork on the tip of the great toes moving proximally depending on the response, respectively [20].

A 15-milliliter phlebotomy was performed under sterile conditions for each participant. The blood obtained was placed in a cool box of 2 to 8 degrees Celsius and transported to the CHUK laboratory within 24 hours for further processing including the freezing of the sera at minus 80 degrees Celsius until it was used for all the desired tests. A full blood count and red cells parameters were obtained using automated counter model 500i of the XS-series hematology analyzers [21][22]. The serum ferritin, folic acid and vitamin B12 measurements were determined using ECL-based immunoassay technique [23][24], and the C-reactive protein was assayed by immune-turbidimetric assay technique [25] all of them using the cobas 4000 analyzer series.

The analysis of outcome dependent variables used both cut-offs as per the standard WHO recommendations [26] and those from a prior similar study that was done in Jordan [27]:

i. Measurement of serum vitamin B12 levels using Elecsys vitamin B12 kit:
B12 levels below 203 pg/ml (low), 203 – 300 pg/ml (low normal), more or equal to 300 pg/ml (normal)

ii. Measurement of serum folic acid levels using Elecsys folate III kit:
Folate levels below 4 ng/ml (low), more or equal to 4 ng/ml (normal)

iii. Measurement of serum ferritin and C-reactive protein using Elecsys kits.
Ferritin levels below 24 mcg/L for male and below 11 mcg/L for female for subjects with CRP less than 4 mg/L [28], and levels < 30 mcg/L for subjects with CRP ≥ 4 mg/L [29] are consistent with depleted iron stores

Statistical analysis

Stata/MP 13.0 (64 bit, StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA) was used for statistical analysis via cross tabulation, regression and t test as appropriate. Continuous variables are expressed in Mean ± Standard Deviation(SD). The associations of variables to the levels of measured micronutrient were subjected to regression analysis and reported in terms of Odds ratio, 95% confidence interval and p value. The statistically significant p value is set to less than 0.05.

RESULTS

The study demographics revealed that among the 191 participants, 72% were female, with most participants between 18 and 35 years old (57%). Of the 191 participants, 30% of them had been educated at the level of secondary and above. The majority of participants had a normal body mass index (78%) with a significant fraction of subjects found to be underweight (9%) [Table 1]

Table 1: Demographics

	N	Percentage
Sex		
Male	54	28
Female	137	72
Age		
18 – 35	108	57
35 – 65	81	42
> 65	2	1
BMI*, Kg/m²		
< 18.5	18	9
18.5 – 25	148	78
25 – 30	19	10
> 30	6	3
Level of education		
Primary and below	133	70
Some secondary	45	23
Higher education	13	7
Occupation		
Farmer	128	67
Student	9	5
Jobless or sporadic employment	27	14
Shop owner or salaried	27	14

*BMI denotes Body Mass Index

Many study subjects (66.5%) reported that they ate twice daily, while most of them were unable to consume a diet containing animal protein and dairy products at minimum on a once weekly basis **[Table 2].**

Table 2: Dietary assessment among 191 study participants

	N	Percentage
Daily Meals frequency		
Once or less	45	24%
Twice	127	67%
Three or more	19	10%
Food category and frequency of consumption		
Dairy products		
Very rarely or never	78	41%
Minimum of once per month	53	28%
Minimum of once per week	48	25%
Minimum of once per day	12	6%
Animal protein		
Very rarely or never	75	39%
Minimum of once per month	64	33%
Minimum of once per week	47	25%
minimum of once per day	5	3%
Green leafy vegetables		
Very rarely or never	0	0%
Minimum of once per month	1	0.5%
Minimum of once per week	63	33%
Minimum of once per day	127	67%

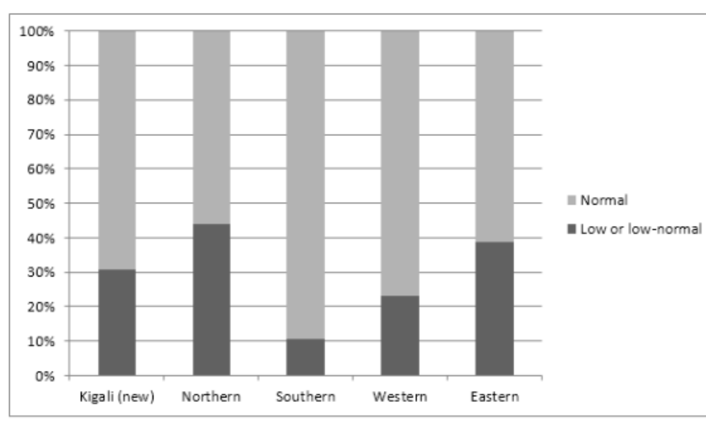
Anemia was observed in 12 (6%) subjects, with the female gender more at risk of subclinical anemia. Among these subjects with anemia, 3 had low normal serum vitamin B12 levels, 2 had iron deficiency and 1 subject had a combined picture of both deficiencies. The prevalence of vitamin B12 deficiency was found to be 10%, while the combined proportion of low and low normal vitamin B12 amounted to 30%. The prevalence of iron deficiency was found to be 4% and serum folic acid levels were normal in the 191 study subjects **[Table 3].**

Table 3: Prevalence of anemia, serum vitamin B12, folic acid and iron def

	Criteria	N	Percentag
ANEMIA			
Male (n=54)	Hemoglobin < 13 g/dl	3	5.5%
Female (n=137)	Hemoglobin < 12 g/dl	9	6.6%
Total		12	6.3%
VITAMIN B12			
Deficiency	B12 levels < 203 pg/ml	19	9.9%
Low-normal	B12 range 203 – 300 pg/ml	39	20.4%
FOLIC ACID			
Deficiency	Folic acid < 4 ng/ml	0	0%
IRON			
Deficiency	For CRP ≤ 4 mg/ml: Ferritin < 24 mcg/L (M) Ferritin < 11 mcg/L (F) OR For CRP > 4 mg/l: Ferritin < 30 mcg/L (any gender)	7	3.6%

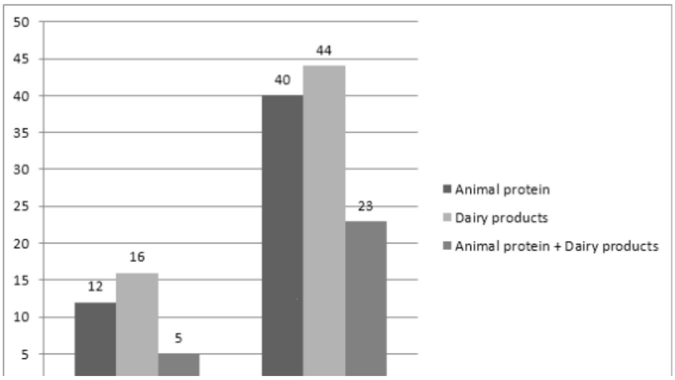
The study population resided in five different regions of Rwanda. The prevalence of low combined with low-normal serum vitamin B12 ranged from 10 to 43% across the regions, with the Northern Province being the most affected area **[Figure 1].**

Figure1: Geographical distribution of low or low-normal vitamin B12



In the category of low and low-normal serum vitamin B12 (total of 58 subjects), only 12 subjects reported they ate animal protein at minimum once weekly versus 16 who reported that they consumed dairy product at that same frequency (Figure 2). By combining the two sources of nutritional vitamin B12 via the scoring method as mentioned in our methods section, only 5 subjects could meet the frequency of a minimum once weekly (equivalent to a nutritional B12 score ≥ 4) versus 23 observed in the group of normal serum B12 levels.

Figure 2: Dietary intakes of natural sources of vitamin B12 at a minimum of once weekly and measured serum vitamin B12 levels



We observed that the age group most affected by low serum levels of vitamin B12 is between 18 and 35 years-of-age, and there is no gender preference. The effect of chronic alcoholism or medications that are notoriously known to impair the normal absorption of vitamin B12 was non-existent. There is a significant association of low or low normal vitamin B12 with history of gastritis/ dyspepsia (OR 1.89, 95% CI [1.01 – 3.53], p = 0.04) though we did not evaluate further the association. There was no association with the history of parasitic infestation or the frequency of consumption of natural sources of vitamin B12 [Table 4].

Table 4: General characteristics and low or low-normal vitamin B12

	Low or low-normal B12 (n = 58) Ratio (%)	OR* [95% CI*]	p-value
Age, years			
18 – 34.9	39/108 (36%)		
35 and above	19/83 (23%)	0.52 [0.27 – 1.00]	p=0.05
BMI*, Kg/m²			
≥18.5	53/173 (31%)		
< 18.5	5/18 (28%)	0.87 [0.29 – 2.56]	p=0.80
Sex			
Male	16/54 (30%)		
Female	42/137 (31%)	1.05 [0.53 – 2.09]	p=0.89
Effect of alcohol			
CAGE* < 2	52/165 (31%)		
CAGE* ≥ 2	6/26 (23%)	0.65 [0.24 – 1.72]	p=0.38
Animal protein			
At minimum once weekly	12/52 (23%)		
Below minimum of once weekly	46/139 (33%)	1.65 [0.79 – 3.44]	p=0.18
Dairy products			
At minimum once weekly	16/60 (27%)		
Below minimum of once weekly	42/131 (32%)	1.29 [0.65 – 2.56]	p=0.45
Animal protein + Dairy products**			
At minimum once weekly	5/28 (18%)		
Below minimum of once weekly	53/163 (32%)	2.21 [0.79 – 6.15]	p=0.13
Presence of past history of			
Parasitic infestation	29/93 (31%)	1.62 [0.58 – 1.99]	p=0.11
Gastritis/ Dyspepsia	34/91 (37%)	1.89 [1.01 – 3.53]	p=**0.04**

*OR denotes Odds ratio, CI denotes confidence interval, BMI denotes body mass index, CAGE denotes the questionnaire used for detection of alcoholism, PPI denotes proton pump inhibitors, Anti H2R denotes histamine 2 receptor blocker. **Refer to nutritional B12 consumption score in the methods section with a cut off ≤ 4.

A comparison of low or low-normal vitamin B12 levels versus normal levels was made (Table 4) and includes the age, the body mass index, gender, effect of alcohol, the consumption of animal protein and dairy product and the presence of a documented past history of parasitic infestation or gastritis/ dyspepsia.

The only significant clinical presentation of low or low-normal vitamin B12 was hyperpigmentation of the knuckles (OR 2.02, 95% CI [1.07 – 3.82], p = 0.03) [Table 5].

Table 5: Neuropsychiatric complaints, skin changes and neurological examination findings

	Low or low-normal B12 (n = 58)	OR* [95% CI*]	p value
Reporting ≥ 3 neuropsychiatric complaints at time of enrollment	18/59 (30%)	1.01 [0.29 – 1.08]	p=1.00
Hyper pigmentation of the skin over the knuckles	27/67 (40%)	2.02 [1.07 – 3.82]	p=**0.03**
Presence of vitiligo	0/6 (0%)	N/A	
Impaired proprioception	6/25 (24%)	0.69 [0.26 – 1.83]	p=0.46
Impaired vibratory sense	2/4 (50%)	2.33 [0.32 – 17.02]	p=0.40

*OR denotes Odds ratio, CI denotes confidence interval

This study reveals that subjects with low or low-normal serum vitamin B12 had the same mean hemoglobin concentration as the subjects with normal serum vitamin B12. Also, there was no significant difference noted in the remainder of complete blood count parameters. [See table 6]

Table 6: C[illegible]e blood count parameters and low or low-normal vitamin B12 Chart Area

	Low or low-normal B12 (n=58)	Normal B12 (n=133)	p value
Hemoglobin concentration*, g/dl	14.2 ± 1.2	14.5 ± 2.5	p=0.18
Mean Corpuscular Volume*, fl	91.6 ± 5.5	91.4 ± 5.7	p=0.6
Red Cell Distribution Width CV*, %	14.2 ± 1.1	14.4 ± 1.4	p=0.14
Red cell Distribution Width SD*, fl	46.3 ± 4.1	46.8 ± 3.9	p=0.17
White cells count*, x10⁹/L	5.4 ± 1.0	5.3 ± 1.5	p=0.69
Eosinophils, %	5.3 ± 4.8	5.8 ± 5.5	p=0.24
Platelets counts*, x10⁹/L	266.1 ± 85.6	226.5 ± 78.3	p=0.99

*values in Mean ± SD

DISCUSSION

Prevalence of anemia: The laboratory investigation documents a prevalence of anemia of 6% in this adult population. This value is comparable to previous surveys done in Rwanda [18], and it is still in agreement to rank this disease entity as a threat of mild public health significance in comparison to the rest of East African block that documented a prevalence of anemia as high as 50% in their healthy populations [19].

Vitamin B12 deficiency: The current study involving 191 adult Rwandans documents a prevalence of vitamin B12 deficiency of 9.9% using the standard WHO definition. This prevalence is just slightly higher than that found in citable population-based studies done in the United Kingdom and the United States which have documented a prevalence of vitamin B12 deficiency ranging from 3-6 % [30][31]. Although this prevalence is much lower than that observed in a similar study done in Jordan, the proportion of marginal vitamin B12 levels remains alarmingly high and should be looked at as a significant public health concern [32]. In our developing world, most of the research work related to B complex vitamins has mainly been spearheaded by the diagnostic work up for cytopenias [4][5] or designed to address health issues affecting specific populations such as childhood malnutrition and psychosis [33][34]. One of the pitfalls encountered whenever the evaluation of these vitamins is tailed to a specific clinical question such as anemia, is the fact that all the B12 deficient states do not always translate in clinically detectable anemia, hence the risk of inaccurate extrapolation of that data [35]. There is still a lack of comparable data on this disease entity among the healthy adult population throughout Africa.

Iron-deficiency: The prevalence of iron deficiency was 4% in our study which was significantly lower than the previous value of 16.5% identified in Rwanda [19], and also much lower than that of a study done in neighboring Kenya which documented randomly selected healthy young individuals and showed a prevalence of 20% for iron deficiency [36]. The continued downward trend in iron deficiency is reflecting the successful implementation of bio-fortified beans across our country since 2014.

Folic-acid deficiency: The normal folic acid levels in this study likely reflects an adequate consumption of a diet rich in natural sources of folic acid such as green leafy vegetables, dried beans, peas, and widely available bananas and its by-products. However, high-risk populations; including the pregnant or lactating women, elderly people, malnourished or individuals on chronic dialysis and alcoholics, are still prone to folic acid deficiency, and the latter are underrepresented in our study. Similar to our findings, one of the citable research works done Africa has also documented a very small prevalence of folic acid deficiency among Zimbabweans with megaloblastic hematopoeisis, a finding that would support that this disease entity is much less prevalent in this geographical location, contrary to the statements in medical textbooks [4].

Even though many of our responders can afford a meal twice a day (67%), the availability of animal protein and dairy products in their diet remains scarce; with 6% consuming dairy products daily, and only 3% who can afford a daily meal that includes animal protein. Although the participants reported a restricted consumption of dairy products and animal protein, this study did not demonstrate any significant association of low or low-normal serum vitamin B12 with dietary habits. This is similar to the finding of the Costa Rican study that evaluated the association of serum vitamin B12 deficiency and dietary intake using standardized food questionnaires, which also was unable to demonstrate a significant correlation [14].

Association with B12 deficiency: In our study, we observed an association between low or low-normal vitamin B12 deficiency with gastritis/ dyspepsia, with the high proportions being seen in the young age groups. Although this finding was not thoroughly evaluated, in our resources limited countries it re-emphasizes the need to orient further our efforts of combating Vitamin B deficiencies [4][31]. We should also turn away from the insignificant proportions of subjects with vitiligo, chronic diarrhea and chronic use of high risk medications that would normally point away from auto-immune process, malabsorption and competitors of vitamin B12 absorption and rule in favor of a nutritional cause as one of the main etiologies of vitamin B12 deficiency as it had been stipulated in several case reports around the sub-Saharan region [33][37]. Until proven otherwise, the lack of intrinsic factor remains a major etiology for vitamin B12 deficiency affecting younger ages as documented decades ago from a Zimbabwean study [4]. However, it should be kept in mind that in countries where basic hygiene and sanitation are still precarious, a seemingly neglected etiology of malabsorption syndrome such as parasitic infestation with giardia lamblia has been cited as one of the causes for vitamin B12 deficiency [12][38].

The nutritional component in the patho-physiology of vitamin B12 deficiency has received the attention of several researchers across the world. In the review of a compilation of 89 fully investigated cases of vitamin B12 deficiency in Russia it was reported that up to 23% of the cases were related to malnutrition while in 16% of the cases there was no identified cause of vitamin B12 deficiency [39]. The association between dietary consumption of vitamin B12 and its deficiency has also been evaluated in the United States and Australia in studies involving refugees from the developing world who were resettling in those countries. One of those studies reported a prevalence of low serum B12 levels of 64% in the pre-settlement versus 27 % in the post-arrival to countries of settlement. The observed high prevalence of vitamin B12 deficiency in the pre-settlement was attributed to having been exposed to a diet depleted in animal protein for decades in their countries of origin [40][41]. On the other hand, a similar study done in Australia involving the same population of refugees has shown that the status of low and borderline serum B12 levels can persist beyond 4 to 8 months, a finding which raises concern about a multifactorial component associated with nutritional vitamin B12 deficiency other than exposure alone to diets poor in that micronutrient [42][43].

Hyperpigmentation in B12 deficiency: In this study, we observed a significant correlation of hyper pigmentation of the skin involving the knuckles in 27 cases out of the 58 subjects with low or low-normal vitamin B12. The skin hyper pigmentation involving the knuckles in association with vitamin B12 deficiency has been documented in some case reports. Though rarely seen, it is thought to be related to the increase in melanin synthesis [7][44]. The most commonly documented areas of hyper pigmentation involve areas that are not necessarily exposed to sun such as the palmar and dorsal aspects of hands or feet, areas of folded skin such as armpit and inguinal creases, tongue, oral mucosa and the skin over the back. In the black population whereby the evaluation of hyper pigmentation might be not obvious, it has been noted in several case reports that the accentuation of hyper pigmentation due to vitamin B12 deficiency is more pronounced on the skin overlying the knuckles [33]. In non-urban settings where advanced laboratory testing remains unavailable, the use of this clinical clue (hyper pigmentation of the skin over the knuckles) in the right context might aid in increasing the index of suspicion for vitamin B12 deficiency.

CONCLUSION AND RECOMMENDATION

This study brings to light the status of serum vitamin B12, folic acid and iron levels in a population of healthy Rwandan adults. Of today, the leading etiology of low serum vitamin B12 in Rwanda remains an unanswered question for future exploration.

We recommend that further research be undertaken with a focus on potential high risk populations such as poor farmers, students in boarding schools and institutionalized individuals like those in jails, who often consume a diet with insufficient intake in animal protein and dairy products. We recommend a dedicated study to evaluate the causal relationship between helicobacter infection and low levels of serum vitamin B12.

The results support that clinicians evaluate chronic cytopenias and transfusion dependent anemia that are often indeed multifactorial, by including the determination of serum levels of micronutrients known to lead to those disease entities especially vitamin B12 which is found to be prevalent across Rwanda.

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Potential Conflicts of Interest (CoI). All authors: no potential conflicts of interest disclosed.

Funding. We are grateful to the University of Utah (USA) for the research grant given to us that has partially covered the expenses encountered during the work related to this research.

Academic Integrity. All authors: confirm that they have made substantial academic contributions to this manuscript as defined by the ICMJE. M.N and F.M conceived and designed the experiment; M.N and B.N performed the experiments including the field work for data collection; B.N coordinated the laboratory measurements; M.N, C.M.M, F.M and V.D critically analyzed the data and participated in the conception of the final manuscript.

Ethics of human subject participation: The study was approved by the local Institutional Review Board. Informed consent was sought and gained where applicable.

Originality: All authors: this manuscript is original has not been published elsewhere

Acknowledgements: we recognize the assistance from Dr. Rosa Canoso, Dr. Kelly Kieffer, Dr. Zubedi Muvunyi, Dr. Louise King, Dr. Tim Walker, Dr. Abdallah J N Utumatwishima, Dr. Marcel Uwimana, Dr. Jean Baptiste Habimana, Dr. Louise Mwiseneza, Dr. Jackline Uwera, Dr. Immaculate Kambutse, Dr. Oswald Habyalimana, Dr. Aloys Tuyizere and Mrs. Kathy in several aspects related to field work arrangements.

Type-editor: Sean Batenhorst (USA)

Review: This manuscript was peer-reviewed by 3 reviewers in a double-blind review process.

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