Profiling vitamin B12 deficiency as an under-recognized significant cause of chronic anemia in Rwanda: the need to re-visit and describe our disease ecology

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ABSTRACT

Background: Chronic anemia poses a significant burden to our emergency room (ER) where affected patients present with low hemoglobin levels requiring immediate blood transfusion. The evaluation of the cause for anemia has been variably undertaken by the receiving physicians. This report aims at providing a preliminary description of the ecology of this disease entity at a tertiary referral hospital in Rwanda.

Methods: Description of complete blood count (CBC) and the status of serum vitamin B12 among randomly selected patients with chronic anemia seen at our Outpatient Clinic (OPD). The effect of empirical supplementation with vitamin B12 is measured by serial CBCs and reticulocyte response in a small series of patients requiring urgent blood transfusion in the ER. Simple descriptive statistics are used to report the findings.

Results: A total of 87 patients are reported in this review. Serum vitamin B12 deficiency was found in 30/87 (34%) subjects with anemia. The affected patients were having significant macrocytosis and reticulocytopenia. The supplementation with vitamin B12 was safe and yielded significant reticulocyte response. We observed a progressive trend up in hemoglobin levels and reversal of the anemia in more than half of the patients when tested in a one-month follow-up. The patients with splenomegaly (thought to be related to malaria) tend to respond less to the supplementation with vitamin B12.

Conclusion: Vitamin B12 deficiency is common among patients with chronic anemia in Rwanda. Empirical supplementation with this vitamin is an attractive and safe option in a clinical setting with a limited laboratory testing capacity.

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

INTRODUCTION

Vitamin B12 deficiency is currently recognized as a worldwide health problem[1]. In developing countries, the proportion of affected young population is large, and often presents in combination with other micronutrient deficiencies [2][3]. In the western world the elderly are more commonly affected by vitamin B12 deficiency [4].

An ongoing, dedicated investigation was opened in 2015 following anecdotal notes from clinical observations that highlighted this problem as a major public health concern [5]. A population based study done in Rwanda has produced evidence that this public health problem has reached alarming proportions; whereby about 10% of the healthy population enrolled in the study was found to have low serum B12 levels in the absence of detectable signs and symptoms (Nkeshimana et al, Thesis 2016).
The fraction of people found with borderline serum levels of vitamin B12 was also significant (figure 1); similar to that documented in other countries [6]. In the remote past, a citable report on the African continent had documented severe presentations of symptomatic anemia due to vitamin B12 deficiency [7], and even in recent years we continue to see similar reports, including unusual presentation associated with this problem such as neuropsychiatric illness and others [8][9].

The etiologies for vitamin B12 deficiency are wide, and currently ongoing studies in Rwanda take into account the possibilities of parasitic infestation, bacterial infections such as tuberculosis, salmonellosis and helicobacter pylori [10]. Some scholars could still hypothesize the rare nutritional deficit as a cause, though current data did not provide any significant correlation (Nkeshimana et al, Thesis, 2016). A combined micronutrient deficiency has been reported as a common health concern in the sub-Saharan Africa [11].

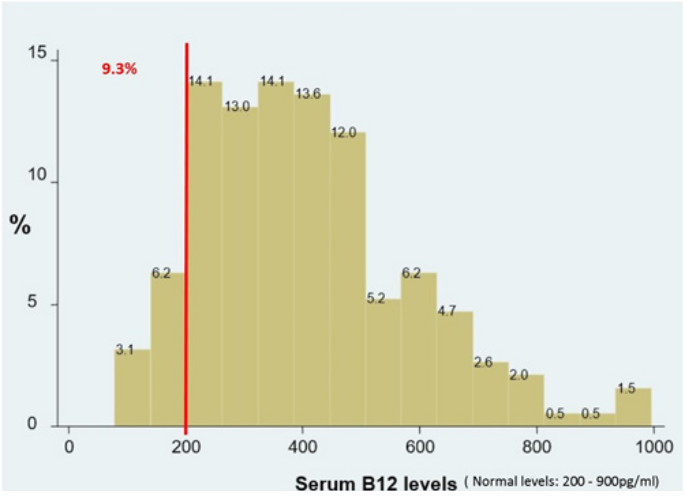

Figure 1: Distribution of serum vitamin B12 levels among a healthy model of Rwandan population. Data from Nkeshimana et al. Thesis 2016

At the Centre Hospitalier Universitaire de Kigali – (CHUK), about 300 individuals per month require blood transfusions. From July 2015 to June 2016, a total of 3342 patients received blood transfusions. A total of 6452 blood pack were used in this period for different clinical indications. (Reference: CHUK Laboratory annual report 2016). The burden of transfusion-requiring anemia at one of the visited provincial hospital in the eastern province of Rwanda was reported to be similarly high, hence the name of "the anemia hill" (translation from local language: agasozi k'indwara y'amaraso make) attributed to one of the neighboring villages where most of the cases are referred from.

In a country with a limited budget for healthcare expenditures, overwhelmed by multiple emergency health concerns to address, it would be a relief to identify the number of vitamin B12 deficient patients among those who receive chronic transfusions. In doing this, the budget allocated to a disease that can be treated otherwise, such as the relation between chronic anemia and vitamin B12 deficiency, could be relocated to address other urgent health issues. This study aims to shed light on this matter, and revolutionize the practice of transfusion medicine at our hospital, and across Rwanda.

METHODS

This is a retrospective chart review of 87 patients who were being followed at the Outpatient Clinic (OPD) for chronic anemia and 7 patients who were treated for presumed vitamin B12 deficiency in our emergency room (ER), and could remain in admission ward 1 of the ER for a dedicated observation of hematological changes during treatment. Regarding the 87 OPD patients, their hospital visits occurred from January through March 2016. We reviewed their demographic characteristics and the laboratory findings documented in their charts (i.e. serum folate, ferritin and vitamin B12 levels). For the 7 patients seen in the ER from December 2016 to March 2017, we reviewed their initial CBCs and controls at weeks 1, 2 and 4 after empirical vitamin B12 supplementation was commenced. Since they received transfusion with packed red cells (and platelet transfusion for one patient) in the ER as per clinical indication, we opted to mainly consider reticulocyte count as the sole marker of therapeutic response to vitamin B12 supplementation. Furthermore, we also reviewed other relevant clinical information that could give a clue on a multifactorial cause of cytopenias in our region (i.e. HIV status, malaria, presence of splenomegaly, creatinine, alanine aminotransferase and lactate dehydrogenase levels).

The anemic patients (including those who had required blood transfusion) reported in this study were 18 years or older at the time this review started. A pre-designed individual questionnaire was used to record all the data reported in this study. CBCs were obtained using an automated counter model 500i of the XS-series hematology analyzers. Biochemical laboratory tests were measured using Cobas 4000 analyzer series. Peripheral thick smears were done for malaria as per clinical indication, and in addition, a special stain (bleu de cresyl) was done for reticulocyte counting. Where applicable, the HIV serological status was determined using the validated national algorithm. At our hospital, the normal serum vitamin B12 levels range from 200 to 900 pg/ml, and a reticulocyte count less than 2% (in the setting of anemia) is regarded as an abnormal bone marrow response. Depending on the medication availability and practicality of the supplementation, oral or injectable forms of vitamin B12 were used in recommended dosage as per clinical guidelines.

RESULTS

The profile of low serum vitamin B12 among patients with chronic anemia

Table 1 Baseline characteristics

		Low Serum B12	Normal Serum B12
		n=30	n=57
1	**Age in Years, Median (IQR)**	36.5 (29 - 45)	37 (25 - 46)
2	**Gender, n (%)**		
	Male	11 (37)	20 (35)
	Female	19 (63)	37 (65)
3	**BMI Kg/m2, n (%)**		
	Less than 18	3 (10)	10 (17)
	18 - 25	19 (63)	42 (74)
	> 25	8 (27)	5 (9)
4	**Household size, n (%)**		
	5 or below	17 (57)	34 (60)
	Above 5	13 (43)	23 (40)

IQR: interquartile range, BMI: body mass index

The majority of patients enrolled in this study are women in their mid-thirties. Most of the study participants had a normal body mass index. There was no significant difference between the family size in both groups of low and normal serum vitamin B12.

The profile of other laboratory parameters in patients with chronic anemia

Table 2 Biological parameters

		Low Serum B12	Normal serum B12
		N=30	N=57
1	**CBC, Median (IQR)**		
	Hb, g/dL	10.9 (9.8 - 11.7)	10.3 (9.5 - 11.8)
	MCV, fL	97.4 (92 - 102)	90.5 (86.1 - 98.1)
	Retic, %	0.89 (0.20 – 1.60)	1.53 (0.20 – 5.0)
	WBC x10^9/L	3.7 (2.9 - 4.5)	3.8 (3.1 - 5.2)
	PLT x10^9/L	170 (71 - 292)	169 (112 - 232)
2	**Serum folate ng/mL, n (%)**		
	4 or below	2 (7)	2 (4)
	Above 4	28 (93)	55 (96)
3	**Serum ferritin mcg/L, n (%)**		
	30 or below	2 (7)	8 (14)
	Above 30	28 (93)	49 (86)

CBC: complete blood count, IQR: interquartile range, Hb: hemoglobin, MCV: mean corpuscular volume, Retic: reticulocytes, WBC: white blood cell, PLT: platelets.

In this study, both reticulocytopenia and macrocytosis trended to be associated with vitamin B12 deficiency. There was no significant difference in hemoglobin levels and other cell lines. The concomitant vitamin B12, folate and iron deficiencies are found to be rare.

Table 3: The therapeutic response to vitamin B12 supplementation at follow-up

No.	Age	Gender	ADMISSION CBC					CONTROL 1		CONTROL 2		CONTROL 3			
			WBC	Hb	MCV	RETIC%	PLT	Hb	RETIC%	HB	RETIC%	WBC	Hb	RETIC%	PLT
1	64	M	2.19	4.4	108	0.2	59	6.7	na	7.1	12.6	4.8	11.7	1.4	258
2	18	M	5.01	8	90	0.6	135	na	na	8.9	25.2	5.46	15.3	na	297
3	47	M	2.92	6.3	90	0.2	74	9	na	10.6	5.4	4.13	13.3	na	379
4	28	F	2.5	3.6	132	na	78	5.1	8.2	6.2	25	2.86	6.8	15.2	101
5	57	M	3.76	3	113	na	67	11.3	na	10.8	na	5.13	16.7	2.6	246
6	27	M	2.53	7	110	2.2	7	8	4	na	na	na	na	na	na
7	59	M	4.84	5.8	110	0.8	68	8.7	0.4	8.9	na	3.76	9.9	na	472

WBC: white blood cell, Hb: hemoglobin, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, RETIC%: reticulocytes percentage, PLT: platelets, na: data not available on electronic records.

From a baseline reticulocytopenia found in the selected index cases, we observe a striking reticulocyte response that could go as high as a percentage of reticulocytes of 25% from 0.6% by the second week post vitamin B12 supplementation. We note that by the 4th week, about 50% of patients had normalized their hemoglobin levels. The same improvement is also observed in other cell lines.

Table 4: Other relevant information

	Blood products used Type & Units	Creat, micromol/L	HIV serology	Malaria smear	ALAT, IU/L	Ferritin, micromol/L	LDH, IU/L	Other
1	2 units PRBCs	41	NEG	NEG	53	1334	na	nil
2	none	na	na	na	na	na	1802	nil
3	2 unit PRBC	82	na	NEG	56	na	na	nil
4	3 units PRBCs	49	NEG	NEG	10	na	1396	Splenomegaly III
5	3 units PRBCs	67	POS	na	52	na	na	nil
6	5 units PLTs	73	NEG	NEG	14	na	509	Giardia Lamblia, Splenomegaly III
7	2 units PRBCs	90	POS	NEG	25	na	2715	nil

PRBCs: packed red blood cells, PLTs: platelets, Creat: creatinine, HIV: human immunodeficiency virus, ALAT: alanine aminotransferase, LDH: lactate dehydrogenase, NEG: negative, POS: positive, na: data not available on electronic records.

The patients who were tested for malaria were found to be negative. In this review, we note that two patients with an enlarged spleen responded to a lesser degree to the empirical vitamin B12 supplementation.

DISCUSSION & CONCLUSION

Micronutrient deficiency, also known as hidden hunger, is a major threat to the world [1] and is common in the adult Rwandan population (Nkeshimana et al, Thesis 2016). Among patients with chronic anemia, who often require blood transfusions in our emergency room, we found a high proportion of underlying vitamin B12 deficiency that was completely reversible with empirical supplementation. This is similar to observations made in other African countries [7][11].

Although chronic anemia had been reported as a rather lesser public health concern in Rwanda [12], especially in comparison to the rates found in other east African countries (MoH report by Erick Boy et al, 2014), the logistics associated with preparation and administration of blood products at the rates of our tertiary care level (more than 6000 packed red cells used per year, CHUK Laboratory Annual report 2015 - 2016) is a cause for concern and should enhance attention to the identification of vitamin B12 deficiency, particularly in patients we treat and follow for cytopenias.

Among the predictive laboratory parameters, we document that the presence of reticulocytopenia is likely to be associated with vitamin B12 deficiency even though the mean red blood cell corpuscular volume might not be as high as described in medical textbooks. Interestingly, this study documents a fraction of 63% (19/30) patients with low vitamin B12 that were found with a mean corpuscular volume below 100 fL, a finding which is one of our key take home messages: a normal MCV does not always mean a B12 sufficient state. As previously documented in other developing countries, this can be explained by a combined deficiency that lead to a self-balanced value of MCV especially when the patient has combined deficiencies leading to both micro and macrocytosis [13] [14]. The deficiency in vitamin B12 seems to have neither gender preference, nor any association with the family size. The fact that it affected the young age should raise concern

that all possible etiologies including the infections, nutritional or occult autoimmune pathology should be evaluated. Considering prior reports on this aspect, we recognize that several infectious processes notoriously known to lead to vitamin B12 deficiency are still endemic in our country.

The laboratory testing capacity is different at each healthcare facility and the patients themselves (or their insurance schemes) might not be able to pay for the additional tests that are necessary to identify the cause for their cytopenias. These facts have often led to repetitive transfusion. As temporary measures, the treating clinicians might consider a therapeutic trial of vitamin B12 supplementation and monitor their response during follow ups at the local clinic. For a sizable number of patients, the effect might simply be impressive and lifesaving. Moreover, this alternative would dramatically reduce the risks associated with blood transfusion and allow the healthcare system to save money by avoiding the costs involved in preparation and administration of blood products for a disease that can be treated otherwise. In addition, the admission frequency in the emergency room for acute severe anemia might be reduced to the minimum necessity. Considering the sample size for the data provided by this study, we acknowledge the limitations that could come along, such as over or underestimation of the fraction of vitamin B12 deficient patients among Rwandans suffering from chronic cytopenias at our hospital. The same is true for such studies that rely on a retrospective review of patients' charts. Due to lack of information and convenience sampling in the ER, we had only a few matching samples to assess therapeutic response to vitamin B12 supplementation; hence, we could not run a paired-sample test to see if the observed effect size is statistically significant. However, it is worth mentioning that the most important factor is the clinical response.

Based on this paper, we also advocate for "a chronic anemia registry" to be introduced at all tertiary care levels for better monitoring of this disease ecology and for defining policy-impacting recommendations in the practice of transfusion medicine. The fact that vitamin B12 deficiency did not trend to be associated with low body mass index and large family size, is a finding that would argue against a nutritional deficit as a major cause, at most, it can be thought of as a co-factor. Dedicated and controlled studies to evaluate all the possible etiologies for this disease entity are highly recommended, and their findings will guide definitive cause-targeting treatment modalities.

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