Original article

Clinical Appropriateness of Serum Folate ordering pattern in a tertiary care hospital in Saudi Arabia

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ABSTRACT

Folate, also known as vitamin B9, is a co-factor necessary for DNA synthesis. Folate deficiency is associated mainly with hematological findings including megaloblastic anemia and pancytopenia. Many countries have mandated grain fortification with micronutrients including folic acid resulting in a reduced prevalence of folate deficiency. Saudi Arabia imports most of the grain products and folate is usually added after milling. There are no local studies to address the folate deficiency prevalence. In this study we aimed to analyse the clinical appropriateness of ordering practice of serum Folate level.

Method: We reviewed all serum folate requests received at our laboratory in Aseer Central Hospital over one-year period (July 2018 June 2019). We collected patients' demographics from the electronic requests along with biochemical results of serum B12, ferritin and CBC results. We assessed appropriateness of orders against pre-specified criteria and applied statistical tests to explore for any association or significance.

Results: Serum folate requests from 614 patients were received during the study period. Serum B12 (543, 88%), and serum ferritin (511, 83%) were concurrently requested. The most common reason for request, when available, was anemia. Anemia was present in (313, 51%) of the subjects for which microcytic anemia was predominant (199, 63.5%), followed by normocytic anemia (101, 33%) and only 10 subjects had macrocytic anemia (3.2%). The most common hematinics' deficiency was ferritin (30%) followed by B12 (17.2%). Serum folate deficiency was low, observed in only 2.8%. Low folate levels were not significantly different between the group with anemia and the normal hemoglobin group.

Conclusion: This study identifies a commonly inappropriate serum folate ordering practice that includes ordering all hematinics at the same visit without considering the possible anemia etiologies. The excessive requests might be related to doctors attempt to avoid multiple blood extractions and to try to reduce the time for diagnosis. These policies are generating unnecessary costs and time loss. Education, phasing out or restricting some tests and introducing laboratory policies like sample storing could help reduce unnecessary requests.

1. Introduction

Folate, also known as Vitamin B9, is a co-factor necessary for DNA synthesis (Chan et al., 2013). Folate belongs to the B group of vitamins and it is present in vegetables and liver. When folate levels are deficient, megaloblastic anemia can develop which is characterized by a poor maturation of red blood cell lineage leading to an increase in immature, large and abnormal erythroblasts in the bone marrow (Chan et al., 2013). Decrease in folate levels can be caused by multiple factors, like insufficient intake, malabsorption, medications or increase in vitamin requirements in con-

ditions like pregnancy, hemolytic anemia and exfoliative skin conditions (Ebara, 2017). Folate deficiency has been implicated in the pathogenesis of neural tube birth defects and several other conditions including cancer and cardiovascular diseases (Pieroth et al., 2018; Li et al., 2016).

The recommended dietary intake has been established as 240 μg/day in people over 18 years old and the requirements are higher for pregnant women up to almost 400 μg/day (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline, 1998). Since 1998, many countries started flour fortifications with folate to prevent neural tube defects (NTD). Currently many countries, including Saudi Arabia mandate flour fortification with micronutrients including folic acid (Food Fortification Initiative, 2019). This has led to global reduction of folate deficiency and some associated conditions like neural tube defects.

Essaying serum folate is needed to explore possible deficiency that might explain symptoms associated with anemia (Devalia et al., 2014). In countries where flour fortification is mandatory, it has been suggested that assessing folate levels is no longer useful and wastes time and money (Brown et al., 2011). In fact, a previous report showed that restriction of red blood cells folate requests significantly reduced costs without affecting the diagnosis of folate deficiency (Shojania and von Kuster, 2010).

Assessing Serum concentrations of folate is the most appropriate initial test to assess folate deficiency (Devalia et al., 2014). However; this laboratory test shows overall low sensitivity and specificity leading to the use of other blood parameters like red blood cell folate and some biomarkers like homocysteine (Sobczynska-Malefora and Harrington, 2018). Hematological indices have also been proposed for folate and vitamin B12 deficiency diagnosis. Unfortunately, no cut offs have been established to define clinical and subclinical states and a wide variety among tests makes it necessary for local reference ranges to be set up (Devalia et al., 2014).

It is also suggested that most of serum folate test requests do not provide useful information and are needless. Healthcare providers are currently using quality indicators to assess cost-effectiveness of medical interventions including utilization of laboratory services (Shahangian and Snyder, 2009). Laboratory based clinical audits in healthcare services are important for quality improvement and are helpful when it comes to giving feedback to laboratory users that should ideally rely on evidence-based practice (Erasmus and Zemlin, 2009). Simultaneously, healthcare facilities are performing audits to reduce costs and waste. It has been reported in many hospitals that requesting patterns usually resulted in unnecessary test requests and, therefore, avoidable costs and workload. The main factor associated with indiscriminate ordering pattern include doctor-related bias and younger doctors with less experience would tend to request more unnecessary tests (MacMillan et al., 2018).

Some research studies performed in countries of the middle east showed that the prevalence of folate deficiency has been found to be on higher side (Azimi et al., 2019). It has been suggested that Saudi Arabia shows high prevalence of folate deficiency, however no epidemiological studies have been performed to our knowledge. Grains are mainly imported to the country and fortification with micronutrients are done after milling. Currently there are four milling companies in Saudi Arabia. Saudi grains organization have issued quality guidelines that mandate wheat flour fortification with micronutrients including folic acid (Quality specification, Saudi Grain Organization (SGO), 2020). Saudi Arabia demographics indicate higher rates of pregnancy and hereditary hemolytic anemia which could contribute to higher prevalence of folate deficiency.

Due to current practice of food fortification in Saudi Arabia, we assumed low folate deficiency prevalence and thus less need for clinical folate testing. The aim of our study is to evaluate folate ordering practice in a tertiary care hospital in Saudi Arabia. We also thought that the result of this study might indirectly shed light and estimate the overall prevalence of folate deficiency in the larger Saudi population.

2. Material & methods

This retrospective study was performed in a tertiary care hospital (Aseer central Hospital, Abha) from Saudi Arabia after approval by the Institutional ethics committee (ACH IRB no. 20190911). All serum folate ordered over the period of 1 year (from July 2018 to June 2019) were analysed. Results of hematological parameters, serum folate, iron, ferritin, and vitamin B12 blood levels were registered from the electronic health records. Likewise, patient information was collected (sex, age, diagnosis, etc.) when recorded in the electronic request form. Samples are either received from inpatient or outpatient clinics.

A complete blood count was assessed through a cell counter (Sysmex 2100,) Electrochemiluminescence immunoassays for ferritin, folate and vitamin B12 were performed in a Cobas e601 auto-analyser (Roche, Basel, Switzerland)

Since suspected folate deficiency is associated with hematological findings, we use the following arbitrary criteria to define appropriate folate order:

- Presence of macrocytosis assessed by mean corpuscular volume (MCV) > 100 μm^3 with and without anemia.
- Presence of bicytopenia or pancytopenia indicated by combination of a white blood cell count (WBC) below 4000 cell/mm^3, Hb below 13 g/dL and 12 g/dL for male and female patients respectively and platelet (PTL) count below 100,000 cell/mm^3.
- Any unexplained Anemia when other hematinics (serum ferritin and B12) are normal.

Low serum folate is defined as level <10 nmol/L. Low serum vitamin B12 is defined as level <179 pmol/L and low serum ferritin as level <15 ng/ml. The reference range values for serum folate, B12 and ferritin are manufacturer generated and are verified to our populations using donor samples . The definition of anemia for adult males (Hb < 13 g/dL), adult non pregnant females (<12 g/dL) were in keeping with WHO definition of anemia (WHO, 2011).

2.1. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 21 (San Diego, CA, USA). For association studies and significance between proportions a Chi-square test was carried out and a p value below 0.05 was considered significant.

3. Results

Over the period of the study, serum folate samples were requested for 614 patients. The mean ages of patients were 40.9 years (40.9 ± 19.3) of which 63% were female. Serum B12 (543, 88%), and serum ferritin (511, 83%) were requested concurrently.

The serum folate level was low (<10 nmol/L) in 2.8% of the patients. 17.2% showed low vitamin B12 levels (below 179 pmol/L) and serum ferritin was low in 155 (30%). Anemia as per WHO definition was present in (313, 51%) of the subjects of which microcytic anemia was predominant (199, 63.5%), followed by normo-

cytic anemia (101, 33%) and only 10 subjects had macrocytic anemia (3.2%). Other hematological abnormalities were depicted on (Fig. 1).

Among patients that presented with anemia over 50% were >40 years old and were predominantly female. It is noticeable that only 3.5% of patients that presented with anemia exhibited low levels of folate and low folate was no different between the anemia and non anemia groups. There was no correlation between these findings, suggesting that anemia patients presented with other types of anaemia (p > 0.05). Similarly, vitamin B12 serum levels were detected in 15% of the patients with anaemia and no association was detected regarding these results (p > 0.05, Table 1).

Ferritin was assessed in 511 patients from which 155 patients (30%) showed levels below 15 ng/ml (mean value 6.9 ± 0.2 ng/ml). Mean folate serum levels were 27.3 ± 0.5 nmol/L. Patients who have low levels of folate (17 patients, mean results 8.15 ± 1.54 nmol/L) showed no significant associations with low levels of vitamin B12, high MCV, high RDW, low PLT or WBC, nor Pancytopenia (p > 0.05, Table 2).

3.1. Folate requests analysis

Considering that an appropriate folate analysis request includes presence of macrocytic anemia, Pancytopenia or bicytopenia, we observed that less than half of folate requests (45.8%) were in accordance to folate ordering appropriateness criteria (Fig. 2).

Macrocytosis was only observed in 1.6% of the patients, indicating that folate request appropriateness would not be fulfilled. Considering all combination of cytopenia (bicytopenia and pancytopenia), there are still >50% where there is no reasonable indication for folate ordering. Although we define normocytic anemia (associated with normal serum ferritin and B12) as an appropriate reason to order folate, most of these are probably anemia of inflammation, a common anemia amongst inpatients. Overall, considering the exceptionally low rate of low serum folate, most of the folate requests might not have been requested appropriately (Fig. 2).

The reason for request information was only available for 173 cases. The main reason for request was anemia (45%) and the analysis results showed that ferritin levels were below 15 ng/ml in 155 (30%) of the total cases and 119 (44.2%) of patients with anemia indicating that the main diagnosis was iron deficiency anemia.

4. Discussion

In this study we assessed the appropriateness of serum folate ordering in a convenient sample at a tertiary hospital. We obtained the laboratory and clinical data – when available - of patients that had folate assessment requests and we tried also to estimate the prevalence of folate deficiency in this targeted population. We also analysed whether folate medical orders resulted in a deficiency diagnosis. We noticed a general pattern of a package ordering – ordering serum ferritin, B12 and folate at the same time to evaluate cases of anemia and we observed that most orders were probably not appropriate. Although ordering folate and B12 together seems appropriate as both deficiencies result in similar clinical manifestations, serum ferritin is requested to investigate a different type of (microcytic) anemia. At least 50% of our samples have microcytic anemia and none of them have been found to have folate deficiency. This suggests a package-based ordering pattern rather than clinically oriented based on anemia features and pre-test probability.

We observed that only 2.8% of the tested patients revealed low folate serum levels. Azimi et al. (2019) reported a higher prevalence of folate and vitamin B12 deficiency in healthy adults (sample of 95 healthy adults) from Iran reporting low serum concentrations of folate and vitamin B12 in 16.8% and 6.1% of the patients, respectively. The authors reported that hemoglobin concentration, but not MCV, correlated with folate and vitamin B12. In our study very few patients presented with macrocytosis and this would be related to inadequate medical requests (discussed below).

We did not find any association between anemia and folate or vitamin B12 deficiency within our study population. This can be explained by the inappropriate ordering pattern (order is not indicated because the pre-test probability is low), and deficiency was not expected based on the picture of the anemia. We also did not find any correlation among low folate or vitamin B12 levels and MCV, RDW, PLT, WBC nor Pancytopenia.

Some authors noticed that some requesting patterns resulted in unnecessary test requests and therefore unnecessary costs and workload and that younger doctors with less experience would tend to request more unnecessary tests (Whiting et al., 2007). A study from Italy showed that 10–20% of folate and vitamin B12 requests would be inappropriate among 466,000 requests from a 3-year period, which is lower than the one reported in our study. The authors proposed that the main approach to solve this problem
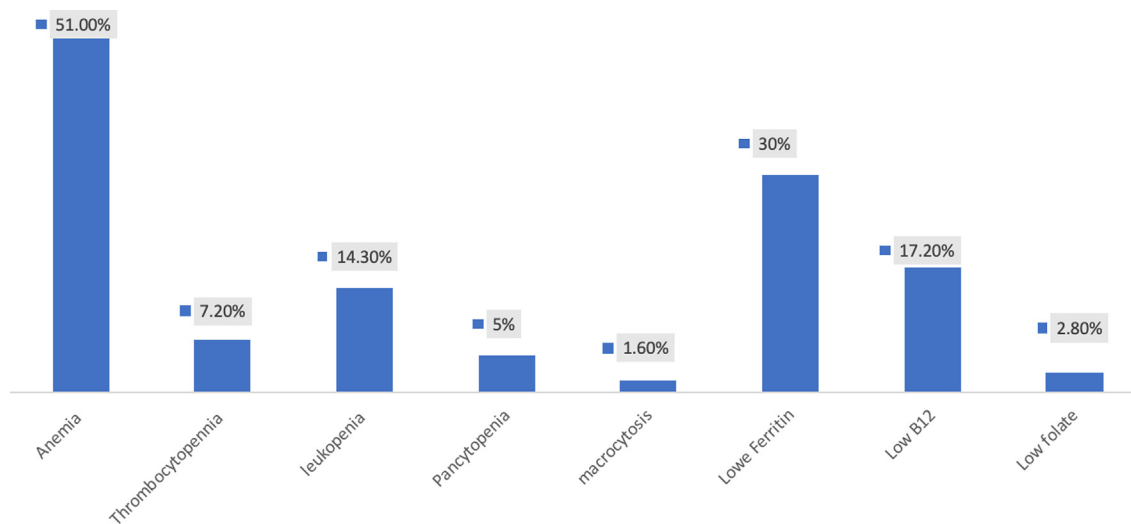

Fig. 1. Abnormal hematological parameters among all cases.

Table 1
Epidemiological characteristics of included cases reference to their anemia status.

Characteristics		Total	Anemia				P value
			N0		Yes		
		No (%)	N0	%	No	%	
Age in years	<20 years	57 (9.3%)	30	9.9%	27	8.7%	0.043*
	20-	139 (22.6%)	87	28.8%	52	16.7%	
	30-	162 (26.4%)	98	32.5%	64	20.5%	
	40-	85 (13.8%)	32	10.6%	53	17.0%	
	50+	171 (27.9%)	55	18.2%	116	37.2%	
Gender	Male	228 (37.1%)	114	37.7%	114	36.5%	0.886
	Female	386 (62.9%)	188	62.3%	198	63.5%	
Folate abnormality	No	597 (97.2%)	296	98.0%	301	96.5%	0.883
	Yes	17 (2.8%)	6	2.0%	11	3.5%	
B12 abnormality (n = 543)	No	341 (82.8%)	152	80.4%	189	84.8%	0.802
	Yes	71 (17.2%)	37	19.6%	34	15.2%	
Ferritin level (n = 511)	Low	155 (30.3%)	36	14.9%	119	44.2%	0.001*
	Normal	356 (69.7%)	206	85.1%	150	55.8%	
WBC abnormality	No	526 (85.7%)	280	92.7%	246	78.8%	0.036*
	Yes	88 (14.3%)	22	7.3%	66	21.2%	
PLT abnormality	No	570 (92.8%)	296	98.0%	274	87.8%	0.047*
	Yes	44 (7.2%)	6	2.0%	38	12.2%	
MCV	Microcytosis	270 (44.0%)	71	23.5%	199	63.8%	0.001*
	Normal	334 (54.4%)	231	76.5%	103	33.0%	
	Macrocytosis	10 (1.6%)	0	0.0%	10	3.2%	
RDW	< 15	332 (54.1%)	261	86.4%	71	22.8%	0.001*
	> 15	282 (45.9%)	41	13.6%	241	77.2%	
Pancytopenia	No	589 (95.9%)	302	100.0%	287	92.0%	0.448
	Yes	25 (4.1%)	0	0.0%	25	8.0%	

P: Pearson X^2 test.
* P < 0.05 (significant).

Table 2
Epidemiological characteristics of included cases reference to their folate status.

| Characteristics		Folate abnormality				P-value
		No		Yes		
		No	%	No	%	
Age in years	<20 years	57	9.5%	0	0.0%	0.039*
	20-	136	22.8%	3	17.6%	
	30-	160	26.8%	2	11.8%	
	40-	80	13.4%	5	29.4%	
	50+	164	27.5%	7	41.2%	
Gender	Male	219	36.7%	9	52.9%	0.033*
	Female	378	63.3%	8	47.1%	
B12 abnormality (n =	No	334	82.9%	7	77.8%	0.078
	Yes	69	17.1%	2	22.2%	
Ferritin level (n = 511)	Low	153	30.9%	2	12.5%	0.077
	Normal	342	69.1%	14	87.5%	
WBC abnormality	No	514	86.1%	12	70.6%	0.046*
	Yes	83	13.9%	5	29.4%	
PLT abnormality	No	555	93.0%	15	88.2%	0.045*
	Yes	42	7.0%	2	11.8%	
MCV c	Microcytosis	262	43.9%	8	47.1%	0.196
	Normal	326	54.6%	8	47.1%	
	Macrocytosis	9	1.5%	1	5.9%	
RDW c	< 15	328	54.9%	4	23.5%	0.001*
	> 15	269	45.1%	13	76.5%	
Pancytopenia	No	574	96.1%	15	88.2%	0.057
	Yes	23	3.9%	2	11.8%	

P: Pearson X^2 test.
* P < 0.05 (significant).

would implicate introducing informatics technology tools as well as education programmes to increase the awareness among clinicians, improving diagnostic tests requests appropriateness and other studies have confirmed its effectiveness (Lanzoni et al., 2017).

Some studies revealed that applying guidelines and policies of intervention caused a significant reduction of inappropriate requests for laboratory tests (Ismail et al., 2019; Pelloso et al., 2016). Regrettably, guidelines related to folate and Cobalamin evaluation are widely variable and range from recommending tests for screening of some diseases like dementia to restrictive strategies (Devalia et al., 2014; Folate Testing, 2015). Additionally, testing these vitamins can usually lead to false positives and false negatives when using the laboratory normal range, and the variability is increased among laboratories and methods (Sobczynska-Malefora and Harrington, 2018).
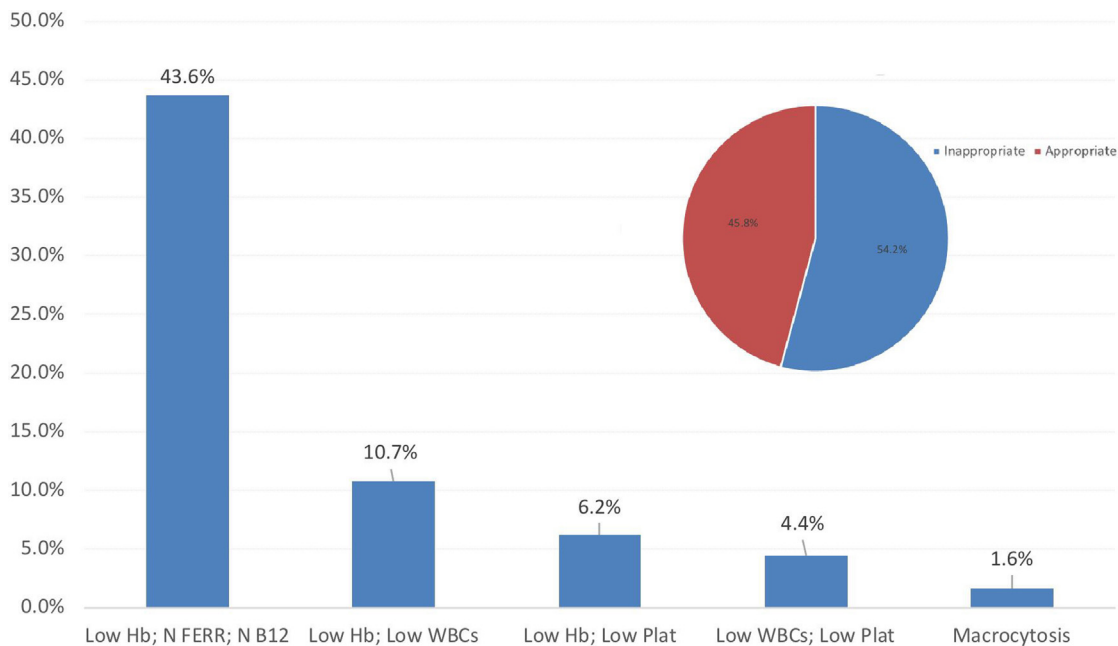
Fig. 2. Conditions for appropriate folate request among sampled cases. 54.2% of requests satisfy none, and thus were deemed inappropriate per per-specified criteria. (Hb: hemoglobin, Ferr: Ferritin, WBCs: white blood counts, Plat: platelets).

Evidence regarding request usefulness in countries with mandatory food supplementation seems to be conclusive, though, and folate and Cobalamin testing is poorly recommended in this setting (Shojania and von Kuster, 2010). Medical requests audit might be the best resource to assure quality care and accurate folate and vitamin B12 testing while helping to improve cost-effectiveness (Shahangian and Snyder, 2009; Erasmus and Zemlin, 2009). Prevalence studies should be carried out in each country and in relation to its demographic characteristics. Ideally, local reference ranges to reduce false outcomes should be performed, and prevalence studies should be carried out especially in those countries that lack food supplementation (Food Fortification Initiative, 2019). In Saudi Arabia, folate food fortification is mandatory, and these quality measures are enforced by penalties and fines from the Saudi grains organization that mandate flour fortification after milling (Quality specification, Saudi Grain Organization (SGO), 2020). Although there are no local studies, folate deficiency prevalence is expected to be low paralleling results from countries with folate fortification (Brown et al., 2011; Shojania and von Kuster, 2010). Homocysteine is a metabolic by-product that has a role in borderline cases and would potentially shed more light (Devalia et al., 2014). It also should be considered that the variations observed in prevalence can be related to variability of cut offs that are used to define vitamin deficiency in addition to geographical and ethnic variations (Willis et al., 2013).

This study presents some limitations. This study is retrospective and the sample is relatively small. Although rare, the ordering physician might be presented with clinical scenario that requires folate testing that was not captured by our retrospective in-design lab records review or our arbitrary criteria for appropriateness. The patients' recent diet, vitamins supplement and the fasting state before blood sampling could not be assessed and captured by our retrospective study which could mask the presence of folate deficiency. Our hospital does not involve maternity and paediatric services and those populations might present different prevalence pattern. Also, it should be considered that laboratory tests for vitamin B12 and folate lack sensitivity and specificity, potentially affecting the conclusions of our study.

5. Conclusion

Present study revealed that serum folate requests from the studied tertiary hospital are being performed in patients that do not fulfil the criteria to suspect this vitamin deficiency and that the main diagnosis arrived is iron deficiency anaemia. The excessive requests might be related to doctors attempt to avoid multiple blood extractions and to try to reduce the time for diagnosis. However, these policies are most probably generating unnecessary costs and time loss. Education of the medical staff would be beneficial for these issues and introducing laboratory policies like sample storing could help reduce unnecessary requests. There is a need for population based epidemiological studies to estimate the true prevalence of folate deficiency in order to inform whether regular testing for folate deficiency is justified or not.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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