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ORIGINAL ARTICLE

Haemato-biochemical basis of anaemia in Agogo, Ashanti Region, Ghana

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This cross sectional study was conducted to evaluate the underlying causes of anaemia among 200 adult patients (18-60 years) without any established chronic disease from September 2011 to February 2012 at the outpatients department of the Agogo Presbyterian Hospital. Participant selection was based on the WHO definition of anaemia in adults [Hb<12.0g/dl (female) and Hb<13.0g/dl (male)]. Venous blood samples were drawn for full blood count (FBC), total iron, ferritin, folate, vitamin B_{12} and malaria parasite tests. One hundred and forty-eight (74%) of the study participants presented with mild anaemia, 40(20%) had moderate anaemia and 12(6%) had severe anaemia. Study participants with mild anaemia presented with higher mean red blood cell count (4.14±0.51 M/μL) when compared to those with moderate (3.71±0.67 M/μL) and severe (2.30±0.73 M/μL) anaemia. About one quarter (51/200) of the study participants had folate deficiency (serum folate <5.0 ng/ml) and 60/200 had vitamin B_{12} deficiency (serum vitamin B_{12}<200ng/L). Iron deficiency (serum iron <8.9 μmol/L), the most prevalent cause of anaemia, occurred in 69/200 of the study participants and had a statistically significant association with the severity of the anaemia (p=0.0028). Malaria infection increased the risk of mild to moderate anaemia (OR=5.2; 95%CI=2.0-13.5) five times and mild to severe anaemia (OR=6.9; 95%CI=1.8-26.9) seven times. Deficiencies in iron, vitamin B_{12} and folic acid are significantly associated with the burden of anaemia in Agogo, Ashanti. It is imperative that the cause of anaemia be fully investigated to enable medical interventions to be fashioned around the underlying aetiology thus optimizing the use of limited health resources associated with the burden of anaemia in Agogo-Ashanti.
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INTRODUCTION

Globally, anaemia persists as a major health problem. The World Health Organization (WHO) estimates that two billion people suffer from anaemia (Underwood, 1996). In populations who are at high risk, anaemia prevalence may be as high as 50-80% with 10-20% presenting with moderate to severe anaemia (World Health Organization, 1992). According to epidemiological data from developing countries, estimated prevalence of anaemia is 39% in children under 5 years, 48% in children 5-14 years, 425 in women 15-59 years, 30% in men 15-59 years and 45% in adults above 60 years (WHO *et al.,* 2001). In a WHO report published by De Benoist et al, (2008), the prevalence of anaemia among non-pregnant women in Africa and South East Asia was estimated to be 45-48% in sharp contrast to 17-19% in the United States and Europe from survey data in 192 member countries spanning from 1993-2005 (De Benoist *et al.*, 2008).

The causes of anaemia are multifactorial. Micronu-

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trient deficiencies of iron, folate and vitamin B_{12} are important causes of anaemia (Provan and Weatherall, 2000). Iron deficiency is the most important cause of nutritional anaemia accounting for almost one million deaths annually (Hercberg and Galan, 1992; Cook *et al.*, 1994). Parasitic infections such as malaria and hookworm have been shown to be associated with anaemia. Studies have established the strong association between chronic anaemia and malaria in malaria endemic areas like Sub-Saharan Africa and Papua (Premji *et al.*, 1995; Hopkins *et al.*, 1997; Guyatt, 2000; Verhoef *et al.*, 2002; Stevens *et al.*, 2013). Chronic intestinal blood loss associated with hookworm infestation and the induced iron deficiency causes anaemia. Despite this global view, little has been done in developing countries by way of identifying the aetiology of anaemia and the contribution of each factor to the burden of the disease.

In Ghana, anaemia in children under 5 years was estimated at 76.1%, 55.9% in non-pregnant women and 62.4% among pregnant women from WHO estimates in 2011 (Stevens *et al.*, 2013). The significant socio-economic impact of anaemia in Ghana makes it imperative to assess the factors contributing to the burden of the disease and hence provide empirical data to support strategies that will aim at reducing the burden of the disease. This study was conducted to evaluate the underlying causes of anaemia in adult patients presenting at the Agogo Presbyterian Hospital in the Asante Akim North Municipality of the Ashanti region.

MATERIALS AND METHODS

Study site and participant selection

This hospital based cross-sectional study was conducted at the Agogo Presbyterian Hospital in the Asante Akim North Municipality from September 2011 to February 2012. The study involved anaemic patients from the Out-patients Department (OPD) between the ages of 18 to 60 years. Pregnant women and persons with documented chronic conditions such as sickle cell anaemia, thalasaemia, malignancy, chronic kidney disease or infections such as tuberculosis, human immunodeficiency virus (HIV) infection and hepatitis B or C were excluded from the study. Five (5ml) millilitres of venous blood was drawn into Vacuette® serum clot activator and K_3EDTA tubes respectively from each participant for full blood count (FBC), malaria parasite, total iron, ferritin, vitamin B_{12} and folate assays. Blood samples in the Vacuette® serum clot activator tubes were centrifuged at 3000 rpm for 5 minutes at room temperature after allowing sufficient time for the sample to clot. The serum samples were aliquoted into Eppendorf® tubes, stored at -80^{0}C and kept away from light. Stool samples were also collected from each participant for screening of intestinal parasites using the sedimentation technique. Ethical approval for the conduct of the study was obtained from the Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST/Komfo Anokye Teaching Hospital, Kumasi and notification to commence the study was given by the CHRPE (CHRPE/184/10) in February 2011.

Sample size estimation

Adjusting for a non-response rate of 2%, with a confidence interval of 95%, the estimated sample size for the study using the formula $N=z^2pq/d^2$ was 200 study participants. Demographic data variables were collected through interview of study participants using a standardized questionnaire.

Laboratory analysis

Full blood count (FBC) was assessed with Sysmex® KX-21N (Sysmex Corporation, Kobe, Japan). Abbot AxSYM® System (Abbot Laboratories, Lisnamuck, Longford, Ireland) was used to measure serum ferritin levels, a useful indicator for body iron stores. Serum iron concentration was measured using the colorimetric method. A solid-phase enzyme immunoassay was employed to detect the concentrations of folic acid and vitamin B_{12} in the serum of study participants (Human Folic Acid ELISA® kit, Human Vitamin B_{12} ELISA® kit, Xiamen, China). Thick (6 µL) and thin (2 µL) blood smears were spotted on microscopic slides using a WHO standardized template and stained with a quality controlled Gurr® Giemsa stain for 10 minutes. The examination of each blood film for malaria parasites was done independently by two

certified microscopist.

Data analysis

Data was analyzed using GraphPad Prism version 6.0 (San Diego California, USA, www.graph pad.com). Data was expressed as mean ± standard deviation (SD). Categorical variables were displayed as frequencies and percentages. Groups of anaemia were compared using chi-square to test for significance at 95% confidence interval. Odds ratio and 95% CI was calculated for the aetiology of anaemia. Micronutrient parameters were expressed as geometric mean (95% CI) and groups compared using one-way ANOVA and Bonferroni pairwise analysis. Relationships between haemoglobin levels, age, haematocrit (HCT), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentrattion (MCHC), iron, ferritin, vitamin B_{12} and folate were established using Spearman rank correlation coefficient.

RESULTS

Out of a total of 200 participants in the study, 40 (20%) were males and 160 (80%) were females. The ages of the study participants ranged from 18 to 60 years and the mean age was 36.9 ± 13.7 years. The 21-30 age group had the highest number of study participants, representing 25% of the study population.

Anaemia Categorization of Study Participants

According to the classification developed by the World Health Organization (World Health Organization, 1968), anaemia was categorized as mild, moderate or severe based on the haemoglobin concentration. Mild anaemia was defined as haemoglobin concentration of 10.0-12.9 g/dL in males and 10.0-11.9 g/dL in females. Moderate and severe anaemia corresponded to haemoglobin concentration of 7.0-9.9 g/dL and less than 7.0 g/dL respectively (Table 1).

Table 1 Personal and demographic characteristics of study participants stratified by anaemia

Personal Characteristic	Total	Mild	Moderate	Severe	
	n=200(n/N)	n=148(n/N)	n=40(n/N)	n=12(n/N)	p value
Age					
≤20	30(15.0)	24(16.2)	14(35.0)	2(16.7)	
21-30	50(25.0)	36(24.3)	12(40.0)	2(16.7)	
31-40	39(19.5)	27(18.2)	10(25.0)	2(16.7)	0.705
41-50	34(17.0)	25(16.9)	6(15.0)	3(25.0)	
51-60	47(23.5)	36(24.3)	8(20.0)	3(25.0)	
Sex					
Male	40(20)	27(18.2)	9(22.5)	4(33.3)	0.412
Female	160(80)	121(81.8)	31(77.5)	8(66.7)	
Marital status					
Married	96(48.0)	70(47.3)	22(55)	4(33.3)	
Single	74(37.0)	55(37.2)	13(32.5)	6(50.0)	0.819#
Divorced	17(8.5)	12(8.1)	4(10.0)	1(8.3)	
Widowed	13(6.5)	11(7.4)	1(2.5)	1(6.3)	
Education					
None	40(20.0)	27(18.2)	9(22.5)	4(33.3)	
Primary	78(39.0)	56(37.8)	11(27.5)	6(50.0)	0.162#
Secondary	66(33.0)	55(37.2)	9(22.5)	2(16.7)	
Tertiary	16(8.0)	10(6.8)	6(15)	0(0.0)	

Data are given as number (percentage) of persons. Percentages are based on totals within each category and may not total 100 because of rounding. Groups are compared using chi-square tests. # indicates p-value when 'moderate' and 'severe' are combined into one group.

One hundred and forty-eight (74%) individuals had mild anaemia, 40(20%) had moderate anaemia and 12(6%) had severe anaemia. The 21-30 and 51-60 age groups each had 36 study participants (36%) with mild anaemia. The 21-30 age group had the highest number of study participants with moderate anaemia. Severe anaemia was more prevalent in the 41-50 and 51-60 age groups, constituting 25% of each age group from Table 1. The mean ages of the participants in the mild anaemia group (38.8 ± 4.1 years) was higher compared to the moderate (36.1 ± 2.0 years) and severe (36.9 ± 1.2 years) anaemia groups.

Red Cell Morphology in anaemia type

Figure 1 shows the absolute red blood cell count (Fig. 1A), mean cell volume (Fig. 1B), haematocrit (Fig. 1C), mean corpuscular haemoglobin (Fig. 1D) and mean corpuscular haemoglobin concentration (Fig. 1E) expressed as mean ± SD. There were significant differences ($p<0.001$) in the red blood cell count of study participants presenting with moderate (3.7 ± 0.7 M/μL) and severe (2.3 ± 0.7 M/μL) anaemia compared with mild anaemia (4.1 ± 0.5 M/μL). The mean MCV of participants within the mild anaemia (82.9 ± 9.3 fL) category was significantly higher when compared with those in

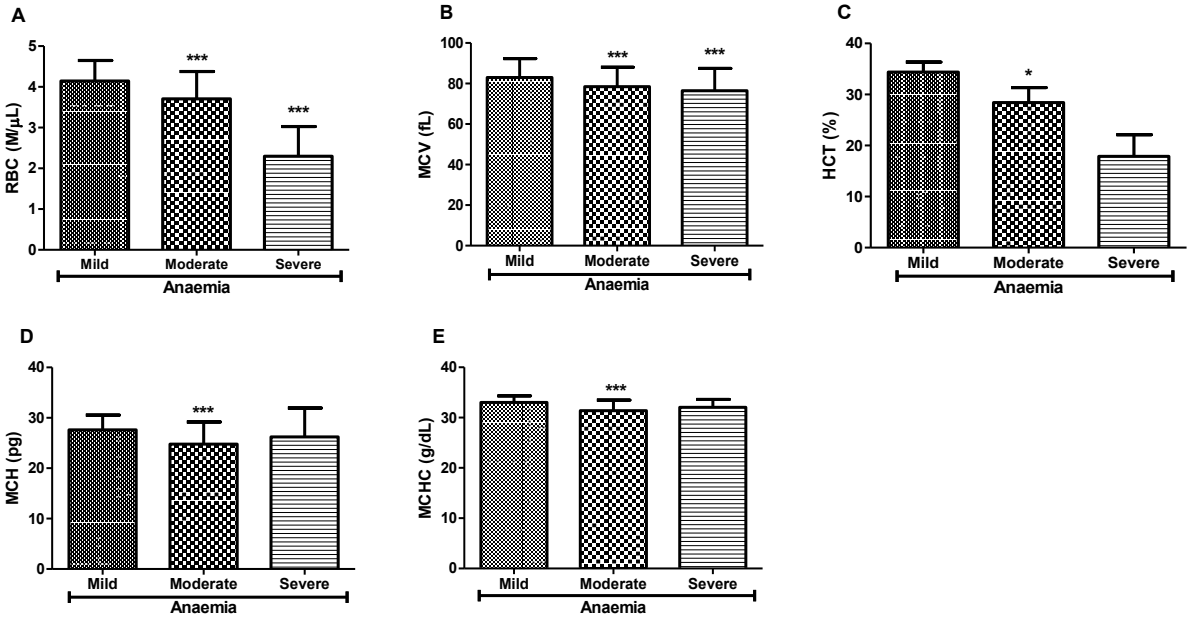

Figure 1 Haemoglobin and red cell indices of study participants stratified by type of anaemia.*=p <0.05, *** =p<0.001: Values significantly different from mild anaemia.

the moderate (78.5 ± 9.6 fL) and severe (76.4 ± 11.0 fL) category ($p<0.001$). The mean haematocrit of moderate anaemia patients (28.4 ± 2.9 %) was significantly lower ($p<0.05$) than those with mild (34.4 ± 1.9 %) anaemia.

The mean haematocrit in the severe (17.8 ± 4.3 %) anaemia category though lower than those in the mild category was not significant ($p>0.05$). The mean MCH (24. 8 ± 4.4 pg) and MCHC (31.4 ± 2.1 g/dl) for participants in the moderate; anaemia category was significantly lower than those in the mild anaemia category (27.6 ± 2.9 pg; 33.0 ± 1.3 g/dL). There was however no significant difference between the mean MCH (25.8 ± 5.7 pg) and MCHC (32.1 ± 1.6 g/dL) of participants in the severe anaemia category compared to those with mild anaemia ($p>0.05$).

From Table 2, 25 (12.5%) of the study participants had P. *falciparum* malaria infestation. Ten (6.8%) of these malaria cases were associated with mild anae-

mia, 11 (27.5%) with moderate anaemia and 4 (33.3%) with severe anaemia. Malaria infection impacted significant risk ($p<0.001$) on the severity of the anaemia. The odds of malaria infection were five times more in moderate anaemia (OR=5.2; 95% CI=2.0-13.5) compared with mild anaemia and seven times more in severe anaemia (OR=6.9; 95% CI=1.8-26.9) compared with mild anaemia. Stool samples of 17 (8.5%) study participants had one or the other of two different intestinal parasites. Fourteen of these stool samples had intestinal flagellates whereas three had hookworm infestation. Red blood cells were present in 9 out of the 200 stool samples received from the study participants. Intestinal parasites did not impact significantly to the severity of the anaemia ($p=0.536$).

Type of Anaemia

The type of anaemia using red cell morphology was segregated using the MCV and MCH. Microcytosis, normocytic and macrocytosis were defined as MCV<80fL, MCV=80-95fL and MCV>95fL respectively. Hypochromasia was defined as MCH<27pg. Of the 200 study participants, 75 (37.5%) had microcytosis, 10(5%) macrocytosis and 115(57.5%) presented with normocytic cells. Hypochromasia was seen in 45.5% of the study participants. Hypochromasia and microcytosis had a significant association with the severity of the anaemia ($p<0.05$). The odds of hypochromasia (OR=2.9; 95% CI=1.4-6.0) and microcytosis (OR=3.1; 95% CI=1.5-6.4) were three times more in the moderate anaemia group compared with the mild anaemia group. Normocytic hypochromasia had no significant association with the severity of the anaemia ($p=0.809$). Microcytic hypochromasia as expected had a significant association with the severity of the anaemia ($p=0.001$) and the odds were four times more in the moderate anaemia (OR=3.5; 95% CI=1.7-7.3) compared with mild anaemia.

In all, 51 (25.5%) study participants had folate deficiency of which 30 (25.0%) had mild anaemia, 13 (32.5%) with moderate anaemia and only 1 (8.3%) presented with severe anaemia. Sixty out of the 200 study participants had vitamin B_{12} deficiency with 47 (31.7%), 8 (20.0%) and 5 (41.7%) in the mild, moderate and severe anaemia categories respectively.

Table 2 Clinical characteristics associated with severity of anaemia.

Clinical characteristics	Total (n=200)	Mild (n=148)	Moderate (n=40)	Severe (n=12)	P-Value	Mild vs Moderate OR(95% CI)	Mild vs Severe OR(95% CI)	Moderate vs Severe OR(95% CI)
Malaria	25(12.5)	10(6.8)	11(27.5)	4(33.3)	0.0002	5.2(2.0-13.5)***	6.9(1.8-26.9)*	1.3(0.3-5.3)
Intestinal parasite	17(8.5)	13(8.8)	4(10.0)	0(0.0)	0.536			
Blood in stool	9(4.5)	5(4.1)	2(5.0)	1(11.1)	0.431#			
Folate deficiency	51(25.5)	37(25.0)	13(32.5)	1(8.3)	0.233			
Vitamin B_{12} deficiency	60(30.0)	47(31.7)	8(20.0)	5(41.7)	0.235	1.9(0.8-4.3)	0.7(0.2-2.2)	0.4(0.1-1.4)
Iron deficiency	69(34.5)	41(27.7)	22(55.0)	6(50.0)	0.003	3.2(1.6-6.6)**	2.6(0.8-8.6)	0.8(0.2-3.0)
Low ferritin	24(12.0)	8(5.4)	14(35.0)	2(16.7)	<0.0001	15(5.6-42.1)***	3.5(0.7-18.7)	0.2(0.0-1.2)
Microcytosis	75(37.5)	45(30.4)	23(57.5)	7(58.3)	0.002	3.1(1.5-6.4)**	3.2(1.0-10.6)	1.0(0.3-3.8)
Macrocytosis	10(5.0)	8(5.4)	2(5.0)	0(0.0)	1.000#	1.1(0.2-5.3)	1.5(0.1-27.8)	1.6(0.1-36.2)
Hypochromasia	91(45.5)	58(39.2)	26(65.0)	7(58.3)	0.010	2.9(1.4-6.0)**	2.2(0.7-7.2)	0.8(0.2-2.8)
Normocytic hypochromic	20(10.0)	16(10.8)	3(7.5)	1(8.3)	0.809	1.4(0.4-5.4)	1.3(0.2-11.0)	0.9(0.1-9.5)
Microcytic hypochromic	70(35.0)	41(27.7)	23(57.5)	6(50.0)	0.001	3.5(1.7-7.3)***	2.6(0.8-8.6)	0.7(0.2-2.7)

Data are given as number (percentage) of persons. Percentages are based on totals within each category. Anaemia groups are compared using chi-square tests. # indicates p-value when 'moderate' and 'severe' anaemia are grouped as one and compared to the mild anaemia group. *p < 0.05, **p < 0.01, ***p < 0.001. OR-odds ratio; CI-Confidence Interval

Cause of Anaemia

Iron deficiency (serum iron <8.9 µmol/L) occurred in 69 (34.5%) of the study participants and this was significantly associated with the severity of the anaemia (p=0.0028). When these 69 iron deficient participants were grouped according to the severity of their anaemia, 41 (27.7%) had mild anaemia, 22 (55.0%) presented with moderate anaemia and 6 (50.0%) with severe anaemic. The odds of iron deficiency in the moderate anaemia group was three times that of the mild anaemia group (OR=3.2; 95% CI=1.6-6.6). Serum ferritin levels less than 12 ng/mL referred to depleted iron stores as defined in the WHO report on serum ferritin concentration for the assessment of iron stores and iron deficiency in populations (Stoltzfus and Dreyfuss, 1998). By the WHO definition, 24 (12%) participants had depleted iron stores, 8 (5.4%) with mild anaemia, 14 (35.0%) with moderate anaemia and 2 (16.7%) had severe anaemia. The depleted iron stores had a significant association with the severity of the anaemia (p<0.0001). The odds of depleted iron stores were 15 times more in the moderate anaemia group compared to the mild anaemia group (OR=15; 95% CI=5.6-42.1). Vitamin B_{12} deficiency, folate deficiency and intestinal parasite infection did not have any significant association with the severity of the anaemia (p>0.05).

Table 3 shows data of the geometric means and 95% confidence interval of iron, vitamin B_{12}, folate and ferritin of all 200 study participants. The table also showed the association between the micronutrient deficiencies and the severity of the anaemia. The data in Table 3 were log transformed to normal, and the geometric means and 95% confidence calculated for each group of anaemia. The geometric mean of ferritin in the mild anaemia group (43.8 ng/mL) was lower compared with the moderate anaemia group (91.8 ng/mL). In the severe anaemia group however, the geometric mean of ferritin was highest (108.0 ng/mL). The trend analysis showed that ferritin levels were associated with the severity of the anaemia (p<0.0001). Similarly, the severity of the anaemia occasioned by the level of serum iron was significantly increased (p<0.0001). The geometric means and 95% CI for the mild, moderate and severe anae-

Table 3 Geometric means of micronutrient parameters of study participants stratified by anaemia

	Total	Mild (A)	Moderate (B)	Severe (C)	P value			
	N=200	N=148	N=40	N=12	All groups	A vs B	A vs C	B vs C
Ferritin	79.9(65.2-98.0)	91.8(74.4-113.2)	43.8(24.5-78.2)	108.0(35.2-331.2)	<0.0001	<0.0001	0.134	<0.0001
Vitamin B_{12}	236.1(211.8-263.1)	222.0(195.1-252.7)	298.1(233.0-358.8)	256.1(155.6-421.3)	0.155	0.058	0.554	0.601
Folate	8.9(7.7-10.2)	9.3(7.9-11.0)	7.2(5.3-9.9)	9.5(5.7-15.8)	0.359	0.162	0.397	0.967
Iron	11.8(10.7-13.0)	13.6(12.3-14.9)	7.7(5.8-10.1)	8.7(4.7-16.0)	<0.0001	0.002	0.233	0.597

Data are presented as geometric mean (95% CI). Groups are compared using one-way ANOVA and Bonferroni pairwise analysis

mia were 13.6 µmol/L (95% CI=12.3-14.9), 7.7 µmol/L (95% CI=5.8-10.1) and 8.7 µmol/L (95% CI=4.7-16.0) respectively. The geometric mean of iron in moderate anaemia category was significantly lower than in the mild anaemia category (p=0.002).

DISCUSSION

Data from the study showed no association between age, gender, marital status and education level with anaemia. From the study, a greater percentage of the study population were female confirming earlier reports of women being more susceptible to anaemia than men (Stoltzfus and Dreyfuss, 1998). In a study conducted at the Ayub Teaching Hospital, Abbottabad, Pakistan, 60.3% of the anaemic study participants were females with 39.71% being males (Idris, 2005). The Abbottabad study differed from this study but confirmed that there were more women with anaemia than men. In another study conducted among University of Peshewar students, women were two to three times more likely to present with anaemia than men (Khan *et al*., 2010b). The results from this study were also consistent with other studies (Karim *et al*., 1994; Paracha *et al*., 1997). The greater susceptibility of women to anaemia may be attributed to the increased requirement of iron due to their monthly blood loss from menstruation and a lower iron intake from food (Cheong *et al*., 1991; Hallberg *et al*., 1995).

The 21-30 and 51-60 year groups combined, constituted 48.5% of the total study participants. A similar study in Ayub Teaching Hospital showed that 40.1% of the anaemic patients fell within these two age groups (Idris, 2005). The 21-30 age group has the physically active individuals undergoing rapid growth and have increased nutritional requirement (Centers for Disease Control and Prevention, 1998; Wharton, 1999). The older adult group (51-60) had an increased incidence of poor nutritional status (Smith, 2000; Choi *et al*., 2004), and may account for why they have a sizeable percentage. Aging has also been shown to physiologically decrease the haemopoietic activity of the marrow (Lipschitz *et al*., 1981).

In this study, 74% of the study participants preseted with mild anaemia, 20% were moderately anaemic and 6% were severely anaemic. In a study of the socio-demographic factors of anaemia in pregnancy in South-western Nigeria, Owolabi and co-workers reported 80.8%, 16.7% and 2.5% for mild, moderate and severe anaemia respectively (Owolabi *et al*., 2012). A report of a study among female students attending the University of Sharjay, UAE put mild anaemia at 88.4%, moderate anaemia at 5.8% and severe anaemia at 4.3% (Sultan, 2007). A study conducted by Mishra and co-workers among 598 females in the Barara village of the Ambala district, India, found 75.5% of the anaemic subjects having mild anaemia, 16.7% with moderate anaemia and 7.8% had severe anaemia (Mishra *et al*., 2012). These results concur with the finding of the present study. Panigrahi and co-workers (Panigrahi and Sahoo, 2011) in their study in Bhubaneswar, Orissa among women in their reproductive age found that of the 146 subjects who were anaemic, 65%, 33% and 2% had mild, moderate and severe anaemia respectively confirming mild anaemia as the most prevalent type, similar to the results of this study. Similarly, Biradar *et al* reported 84%, 15% and 1% for mild, moderate and severe anaemia respectively in a one year cross-sectional study they conducted among adolescent girls in Vantamuri Primary Health Care (PHC)(Biradar *et al*., 2012).

The association between iron deficiency and anaemia and its indication as the most significant contributor to the public health importance of anaemia has been well documented (Agyei-Frempong *et al*., 2001). There is however paucity of data on the prevalence of iron deficiency among adult anaemic patients in Agogo, Ghana. In this study, 34.5% of the study participants were iron deficient. The study further showed that, 12.0% of the anaemic patients had depleted serum ferritin stores. A prevalence rate of 85.7%, more than twice of what this study found was reported among anaemic patients in Phan Tien village, southern Vietnam (Le Hung *et al*., 2005). Idris also reported 68% as the prevalence rate of iron deficiency (Idris, 2005). Umeta and co-workers reported a prevalence rate of 17% compared to the 34.5% reported by this study (Umeta *et al*., 2008). A prevalence rate of 16.5% was reported among adolescent girls in New Halfa,

Eastern Sudan (Abdelrahim *et al.*, 2009). In a study to evaluate the iron status of adults in the capital area of Finland (Lahti-Koski *et al.*, 2003), 16% of the women were observed to have depleted iron stores using their serum ferritin levels as reference, a little higher than the results of this study. The results of this study which indicated iron deficiency as the most significant contributor to anaemia may possibly be attributed to insufficient dietary intake of iron or low dietary bioavailability of iron, malaria and the loss of blood through menstruation.

Vitamin B_{12} deficiency was the second highest aetiological cause of anaemia in Agogo with 30% of the study participants expressing low serum vitamin B_{12} concentration. The possible reason for this level of deficiency could be assigned to inadequate dietary intake especially as this study was focused on young adults without any established chronic condition. Several Jordanian studies (Abu-Samak *et al.*, 2008; Barghouti *et al.*, 2008) have published prevalence rates of between 16 to 50%. In a study in India where there is a low intake of animal source food, 47% of the adults in the study reported with low serum vitamin B_{12} concentration (Refsum *et al.*, 2001). In Nigeria, 9% of 162 girls screened had low vitamin B_{12} concentration (VanderJagt *et al.*, 2000) in contrast to a few children who reported with low vitamin B_{12} serum concentration (Abrams *et al.*, 2003) in Botswana. Yajnik and co-workers reported vitamin B_{12} deficiency of 67% among healthy Indian men (Yajnik *et al.*, 2006), a prevalence rate much higher than the results of this study. Reports of surveys done in Latin America, estimates that approximately 40% of children and adults have deficient or marginal vitamin B_{12} status (Allen, 2004). Low serum vitamin B_{12} concentration has been well documented to be associated with anaemia and altered neurological function (Lindenbaum *et al.*, 1988). Dietary supplementation with meat has greatly improved cognitive performance in Kenyan school children (Whaley *et al.*, 2003) whereas fortfication of food with vitamin B_{12} in the Netherlands has proved very useful. In an earlier study undertaken in the Netherlands (Winkels *et al.*, 2008), participants aged 50-65 years were made to consume bread fortified with 9.6 μg of vitamin B_{12} for 12 weeks. The results indicated that the proportion of participants with low serum vitamin B_{12} reduced from 8% to a remarkable 0%.

In this study, low serum folate concentration was observed in 25.5% of the study participants. Several studies have provided scientific evidence of the importance of folic acid supplementation for the prevention of neural tube defects in women of child bearing age (Vergel *et al.*, 1990; Berry *et al.*, 1999; Vergel *et al.*, 2005; Wilson *et al.*, 2007). VanderJagt and co-workers reported a much lower prevalence rate of 2.4% among female adolescents in Northern Nigeria (VanderJagt *et al.*, 2000). A study of anaemic pregnant females in a tertiary care centre at Rawalpindi reported a folate deficiency prevalence rate of 20%, a little lower than what this study established (Khan *et al.*, 2010a). Folate deficiency of 11.9% was reported among anaemic pregnant women (Marti-Carvajal *et al.*, 2002) whereas in New Halfa, Eastern Sudan, 69% of adolescent girls reported with folate deficiency (Abdelrahim *et al.*, 2009). An equally high prevalence rate was reported by Thoradeniya *et al* in Colombo, Sri Lanka (Thoradeniya *et al.*, 2006).

The results of this study showed that 12.5% of the anaemic adult patients attending the OPD clinic had malaria confirmed by a positive Giemsa stained blood slide. In Buea, Cameroon, a malaria prevalence rate of 21.9% was reported among adults enrolled in the study (Takem *et al.*, 2010) whereas Mayor *et al* (2007) reported a rate of 14.4% with all the parasitaemia reported was attributable to P. *falciparum*. In Sub-Saharan Africa where malaria is endemic, the association between chronic anaemia and malaria is so strong that anaemia is often taken as a proxy indicator of the malaria control programmmes (World Health Organization and Centre for Disease Control and Prevention., 2007). Takem and co-workers demonstrated that by clearing parasites of patients, the burden of anaemia could be reduced by more than one third (Takem *et al.*, 2010). Although this study established a significant association between malaria and anaemia, some studies have reported no association (Stoltzfus *et al.*, 2000).

In this study, 8.5% presented with intestinal parasite infection with only three study participants having hookworm infestation. This study did not detect any association between intestinal parasite infection and anaemia in Agogo similar to what Takem *et al.* (2010) reported.

CONCLUSION

This study has identified that the most significant contributors to the burden of anaemia in Agogo are iron deficiency, vitamin B_{12} deficiency, folate deficiency and malaria. The study identified that gender and level of education did not play any significant role in the development of anaemia although there were more women than men as expected. The multifactorial nature of causes of anaemia requires extensive investigation of patients who present with the condition before blindly treating with haematinics. Upscaling a similar study with a larger population size could prove useful in presenting a national picture on the burden of the disease to further reinforce the need for concerted effort to address the aetiology of anaemia. The socioeconomic cost of anaemia is too high a price to pay especially for a developing country like Ghana.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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