ORIGINAL ARTICLE

Ethnic Disparities in Vitamin B12 and Folate Deficiency Prevalence and their Haematological Correlates in Malaysia.

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Submitted: 07/08/2025. Revised edition: 13/10/2025. Accepted: 22/10/2025. Published online: 01/11/2025.

Abstract

Introduction: Vitamin B12 and folate deficiency are preventable conditions that can lead to serious haematological and neuropsychiatric complications if untreated. Global prevalence varies, and data from Malaysia is limited. This study aimed to determine the proportion and associated risk factors of these deficiencies in a Malaysian hospital population. **Materials and methods:** A cross-sectional, retrospective study was conducted using data from the Laboratory Information System of Hospital Teluk Intan from June 2023 to May 2024. Demographic data and serum B12/folate levels from 1,463 subjects were analyzed. Deficiency proportions were calculated, and associations with demographic factors and haematological profiles were examined using Chi-square and multinomial logistic regression. **Results:** The proportion of isolated vitamin B12, isolated folate, and combined deficiencies were 3.2%, 14.5% and 2.9% respectively. Indian ethnicity was significantly associated with higher odds of all deficiency types. Chinese ethnicity was associated with isolated B12 deficiency. Males and younger age groups (12-19 and 20-29 years) had significantly higher odds of isolated folate deficiency. A high proportion of macrocytic anaemia was observed in isolated B12 (52.17%) and combined deficiencies (64.28%), as was megaloblastic anaemia (56.52% and 61.90%, respectively). **Conclusion:** Significant racial, age, and gender disparities exist in the prevalence of B12 and folate deficiencies, which are strongly associated with haematological complications. These findings highlight the need for targeted screening and intervention strategies in high-risk groups within the Malaysian population.

Keywords: *Deficiency, folate, macrocytic anaemia, Malaysia, Vitamin B12.*

Introduction

Vitamin B12 and folate are involved in various biosynthesis processes, including DNA synthesis, nucleoprotein, erythropoiesis, myelin synthesis, normal growth and cell reproduction and one-carbon metabolism [1-5]. Carmel et al., (1977) mentioned in their study that average total body stores of vitamin B12 are 3–5 mg, and when there is little or no vitamin B12 replenished from the diet, the stores may last for up to 5–10 years before manifestations of vitamin B12 deficiencies are seen clinically [6]. Deficiency can lead to various consequences such as megaloblastic anaemia, cardiovascular complication, impaired cognitive function, neurological manifestations like peripheral neuropathy, subacute combined degeneration of spinal cord, neural tube defects [4,7]. Megaloblastic anaemia is a type of macrocytic anaemia characterized by high mean corpuscular volume (MCV) > 101 fL and mean corpuscular haemoglobin (MCH) >32 pg [2]. It is distinguished from other macrocytic anaemia by the presence of large red cells precursors in the bone marrow due to asynchronous maturation between the nucleus and cytoplasm caused by impaired DNA synthesis [3-5].

The definition of vitamin B12 deficiency varies globally, depending on the country and the biomarkers used [7]. For instance, the United States (U.S) National Institute of Health (NIH) states that most laboratories define deficiency as serum vitamin B12 less than 148 pmol/L and insufficiency as below 221 pmol/L [7]. In contrast, the United Kingdom's (UK) National Institute for Health and Care Excellence (NICE) guidelines recommend serum B12 level of <133 pmol/L as the cut-off of absolute B12 deficiency with level of B12 between 133-258 pmol/L regarded as possible deficiency [8]. Data from the National Health and Nutrition Examination Survey (NHANES) from 2007–2018 found the national prevalence of vitamin B12 deficiency and insufficiency among US adults (aged ≥ 19 years) was approximately 3.3% and 12.5% respectively [7]. In UK, the estimated prevalence of B12 deficiency was 6% [9] with higher prevalence of 11% seen in vegetarians [9], consistent with study by Pawlak [10]. The highest national prevalence was reported in India which ranges between 35% to 47.19% [11- 12].

World Health Organization (WHO) defines folate deficiency and insufficiency as having serum Folate of <6.8 nmol/L and 6.8−13.4 nmol/L respectively [13]. In comparison the United Kingdom National Diet and Nutrition Survey Rolling Program (NDNSRP) recommends a serum folate of <7nmol/L as the deficiency threshold, a level significantly associated with megaloblastic anaemia [14]. An 8-year study at the Mayo clinic (2010-2018) involving 197,974 samples, found that the prevalence of folate deficiency in the general US population to be 0.7%, a drop from 16% in 1996 [15]. Furthermore, data from UK NDNSRP (2009 - 2019) revealed a folate deficiency prevalence of 11% in adults aged 19- 64 years, with higher rates observed in the elderly group (>65 years old) and adolescents (12-18 years) at 13% and 17%, respectively [14]. The literature on the prevalence of folate deficiency among Malaysian population is scarce, with the latest available study dating back to 2006. Khor et al., (2006) reported a prevalence of 15.1% among healthy non pregnant women of childbearing age in Malaysia, while a parallel study in Indonesia found no folate deficiency in their subjects [16]. A more recent 2017 study in India found a folate deficiency prevalence of 12% among healthy urban Indian adult population [12].

According to the WHO, anaemia in adult is defined as haemoglobin (Hb) less than 13 g/dL in men and Hb less than 12 g/dL in women. Approximately 30% of global population is affected by anaemia, with 60% of cases caused by nutritional deficiency [13]. A prospective study done in St Stephen's Hospital, Delhi found that 24% of hospitalised patients had an Hb value <10 g/dl, and 2.7% were identified as having macrocytic anaemia [17]. Khajuria A et al., (2022) found that among patients with Hb <10 g/dl, 20% had macrocytic anaemia, with pure vitamin B12 deficiency accounted for 55% of these cases, while 8% folate and combined deficiencies each

accounted for 8% [18]. Another Indian study by Mahajan et. Al., (2015) found 37% and 23% of patients with B12, folate and combined deficiencies had macrocytosis and megaloblastic anaemia, respectively [19].

Despite abundant studies on the prevalence of B12 and folate deficiency and their associated risk factors in other countries, the data remained limited in Malaysia. The purposes of our study are to determine the proportion of patients with B12 deficiency, folate deficiency and combined deficiencies among patients tested for serum B12 and folate in our laboratory. Additionally, this study aims to explore the association of age, gender and ethnicity with these deficiencies, and to study the correlation between vitamin B12 and folate levels and haematological profiles.

Materials and methods

Study design and sampling

This cross-sectional, retrospective record review study was conducted in Pathology Laboratory HTI from January 2025 to October 2025. Ethical approval was obtained from the Institutional Ethics Committee. The study aimed to estimate the proportion of vitamin B12, folate, and combined vitamin B12 and folate deficiencies among patients tested for B12 and folate. Census sampling was applied. The largest computed sample size from calculations for different deficiency types was selected; a sample size of 382 was computed using a 95% confidence level, an expected proportion range of 8.2% and 47% [11-12], and a margin of error of 5% [20]. These calculations are summarized in Table 1.

Data collection

The data was extracted from the Laboratory Information System (LIS). The extracted information included demographic data (ethnicity, age and gender), serum vitamin B12 and folate values, full blood count (FBC) and full blood picture (FBP) findings, which were recorded in a pre-tested data collection form. The samples processed in our laboratory were sourced from inpatients and outpatients at Hospital Teluk Intan and Hospital Tapah, as well as from outpatients at public health clinics in Hilir Perak, Bagan Datuk, and Batang Padang districts.

Serum B12 and folate were measured using the fully automated Alinity i machine by Abbott Diagnostic. The test utilizes Chemiluminescent Microparticle Immunoassay technology, utilizing intrinsic factor-coated microparticles for B12 quantification and a Folate Lysis Reagent for serum folate quantification. The samples were all analysed in the Pathology Laboratory HTI and the results were uploaded into the LIS.

Inclusion and exclusion criteria

Data from a total of 1,753 subjects tested for serum vitamin B12, folate, or both between 1 June 2023 and 31 May 2024 were recorded. The inclusion criteria were subjects more than 12 years old with complete demographic data. Exclusion criteria were rejected and redundant samples sent within the study period. A total of 1,463 subjects met the inclusion criteria and were included in the final analysis. Out of 1,463 subjects, 1,434 subjects were tested for both B12 and folate, 20 subjects were tested for B12 only and 9 subjects were tested for folate only.

Independent variables

This study obtained demographic data including gender, age and ethnicity from the subjects. These data were analyzed to determine the factors associated with B12 or folate deficiency status. Ethnicity was classified into four categories: Malays, Chinese, Indians and Orang Asli. Age was categorized into eight groups: 12 to 19 years, 20 to 29 years, 30 to 39 years, 40 to 49 years, 50 to 59 years, 60 to 69 years, 70 to 79 years and more than 80 years. Deficiency status was analyzed to find the association with the presence of macrocytic anaemia and megaloblastic anaemia.

Dependent variables

Deficiency status of the subjects was determined by our laboratory cut off values. Our laboratory defines vitamin B12 and folate deficiency using age-specific cutoff values [21]: for serum B12, deficiency is indicated by levels <138 pmol/L in adults (>19 years), <150 pmol/L for ages 17–19, <180 pmol/L for ages 14–17, and <186 pmol/L for ages 12–14. For serum folate, deficiency is defined as <7 nmol/L in adults, <18 nmol/L for ages 14–19, and <27 nmol/L for ages 12–14. Subjects with normal B12 and folate level were classified into four groups: group A (normal) for subjects with normal B12 and Folate level, group B (isolated B12 deficiency) for subjects with B12 level below laboratory cut off value but with normal folate level, group C (isolated folate deficiency) for subjects with folate level below laboratory cut off value but with normal B12 level were classified into isolated folate deficiency group and lastly group D (combined deficiencies) for subjects with both B12 and folate level below laboratory cut off value.

Haematological profile including haemoglobin level (g/dL), mean corpuscular volume (fL) and mean corpuscular haemoglobin (pg) and full blood picture examination were analyzed to find the presence of macrocytic anaemia and megaloblastic anaemia in the subjects. A haemoglobin (Hb) level of less than 13 g/dL in men and Hb less than 12 g/dL in women is defined as anaemia, while macrocytic anaemia is characterized by the presence of anaemia with high mean corpuscular volume (MCV) > 101 fL and mean corpuscular haemoglobin (MCH) >32 pg. The presence of megaloblastic anaemia is defined by the full blood picture findings of presence of macrocytosis and hypersegmented neutrophils with concomitant B12 or folate deficiency.

Data analysis

Descriptive analysis was carried out to characterise the dependent and independent variables. The overall proportion of B12, folate, and combined deficiencies was calculated. The proportion of each deficiency across all categories of the variables was also calculated to find out which group of the subjects show highest proportion of deficiency. Chi square analysis was performed to assess the associations between each demographic predictors with deficiency status, as well as the association between deficiency status with the presence of macrocytic anaemia and megaloblastic anaemia. Multinomial logistic regression analysis was conducted to examine the association between demographic predictors and vitamin deficiency status, categorized as no deficiency, isolated B12 deficiency, isolated folate deficiency, and combined deficiencies. Predictors included gender, ethnicity and age group.

Ethical approval

Institutional Ethics Committee, with Medical Research & Ethics Committee (MREC) approval number 24-03916- 5RH (1), with permission to publish obtained from the Director General (DG) of Health.

Results

A total of 1,463 subjects were analyzed. The cohort consisted of 57.6% (n=842) females and 42.4% (n=621) males. Ethnically, 55.9% (n=818) were Malay, followed by Indian 24.2% (n=354), Chinese 16.9% (n=247) and Orang Asli 3% (n=44). The mean age was 58.1 ± 18.2 years. Of the total subjects, 79.6% (n=1165/1463) had normal B12 and folate (group A), 3.2% (n=46/1454) of the subjects were found to have isolated B12 deficiency (group B), 14.5% (n=210/1443) had isolated folate deficiency (group C) and 2.9% (n=42/1434) had combined B12 and folate deficiency (group D) (Figure 1), with different proportions of deficiency observed across different categories. The median vitamin B12 and folate for all subjects were 427.0 (IQR 266.0, 756.0) pmol/L and 16.9 (IQR 8.4, 37.7) nmol/L, respectively. Statistically significant differences in median B12 and folate level ($p<0.05$) were observed across all groups. The

baseline demographic distributions and median levels are summarized in Table 1.

A higher proportion of isolated B12 deficiency was observed among females, Indian ethnicity, and of age between 60 to 69 and 70 to 79 years old, which were reported as 71.7% (n=33/46), 54.3% (n=25/46), 21.7% (n=10/46), and 30.4% (n=14/46), respectively. In contrast, the proportion of isolated folate deficiency was highest among Malays (53,8%, n=113/210), with no prominent gender or age group differences. For combined deficiencies, a higher proportion was seen among Indians 73.8% (n=31/42), as well as subjects age 70–79 years 26.2% (n=11/42), with no significant gender differences (Table 1).
Chi-square analysis showed significant associations ($p<0.05$) between ethnicity and all three deficiencies with varying strength of associations (Cramer's V = 0.25, 0.18, and 0.20 for B12, folate and combined deficiencies, respectively). Gender and age were significantly associated only with folate deficiency ($p<0.05$), with associations strength of 0.10 and 0.25 (Table 2).
A multinomial logistic regression analysis was performed to identify the effect of gender, ethnicity, and age on the deficiency status, using Group A (normal) as the baseline reference category. Male were 2.22 times more likely to have isolated folate deficiency (OR=2.22, 95% CI [1.65, 2.99], $p<0.05$), but were not significantly more likely to have combined deficiencies (OR=1.74, 95% CI [0.91, 3.32], p=0.09) or less likely to have isolated B12 deficiency (OR=0.94, 95% CI [0.84, 3.25], p=0.13). Compared to Malay ethnicity, Indian ethnicity was associated with higher odds of having isolated B12 deficiency (OR=11.83, 95% CI [6.36, 22.01], $p <0.05$), folate deficiency (OR=2.63, 95% CI [1.91, 3.62], $p <0.05$) and combined deficiencies (OR=15.88, 95% CI [6.09,41.42], $p <0.05$). Chinese ethnicity had only significantly higher odds of having isolated B12 deficiency (OR=4.60, 95% CI [2.17, 9.74], $p<0.05$). Individuals of age 12-19 were 21.09 times more likely (OR=21.09, 95% CI [9.12,48.76], $p<0.05$), and those aged 20-29 years were 3.01 times more likely (OR=3.0, 95% CI [1.67, 5.43], $p <0.05$) to have isolated folate deficiency. No significant effect of age was found in isolated B12 deficiency and combined deficiencies (Table 3).

The haematological profiles of subjects were analyzed, focusing on the presence of macrocytic and megaloblastic anaemia. The proportion of macrocytic anaemia in vitamin B12 deficiency was 52.17% (n=24/46), 8.09% (n=17/210) in folate deficiency, and 64.28% (n=27/42) in combined deficiencies. The proportion of megaloblastic anaemia in vitamin B12 deficiency was 56.52% (n=26/46), 5.70% (n= 12/210) in folate deficiency, and 61.90% (n= 26/42) in combined deficiencies. Chi-square analysis showed significant associations ($p<0.05$) between B12, folate and combined deficiencies with the presence of macrocytic and megaloblastic anaemia with varying strength of associations (Cramer's V = 0.40, 0.13, 0.35) and (Cramer's V = 0.56, 0.10, 0.47) respectively. These findings suggest that B12 deficiency, alone or in combination with folate deficiency, is more strongly linked to macrocytic or megaloblastic anaemia compared to folate deficiency in both conditions (Table 4).

Discussion

The recognition of vitamin B12 and folate deficiency is important as these deficiencies can present with haematological, neuropsychiatric, and other adverse clinical manifestations [1-2]. One of the most commonly observed complications is megaloblastic anaemia, in which is a key focus of our study. Despite the well-known consequences and healthcare burden of these deficiencies there are very few studies focusing on the prevalence of B12 or folate deficiency in the Southeast Asia region, particularly in Malaysia.
The proportion of vitamin B12 deficiency in our study was 3.2%, based on our laboratory

deficiency threshold of <138 pmol/L. This rate is higher than the 3.3% prevalence reported in the US (using a <148 pmol/L threshold) [7], equivalent to the 6% reported in the United Kingdom (using a <133 pmol/L threshold) [9], but significantly lower than the 35-47.19% reported in India (using a <150 pmol/L threshold) [11, 12, 17]. The high prevalence of B12 deficiency in our study was attributable to the large proportion of Indian subjects, who commonly practice vegetarianism. This is consistent with previous studies, linking high B12 deficiency rates across many regions in India to strict vegetarian diets [11, 17-18]. Hunt and Pawlak also found a higher prevalence of B12 deficiency among vegetarians, attributing it to inadequate B12 intake, as the vitamin is synthesized only by microorganisms and is absent from plant-based foods unless fortified [9, 10].

The proportion of folate deficiency in our centre was 14.5% with a deficiency threshold of <7nmol.L, consistent with a 2006 Malaysian study by Khor et al., (2006) which reported a 15.1% prevalence of folate deficiency among healthy women of childbearing age, with a parallel study done in Indonesia found none of their subjects were folate deficient, owing to the mandatory wheat-flour fortification programme in Indonesia [16]. Our prevalence also aligned with the 11% national prevalence of folate deficiency in the UK, with the same level of folate deficiency threshold [14] as well as higher than the national prevalence of 0.7% in the USA. This is attributed to the voluntary basis of folic acid food fortification in the UK compared to the mandatory folic acid food fortification in the US since 1998 [7]. In comparison to other literature from the eastern countries, the prevalence of isolated folate deficiency in our centre is higher than previous study in India which reported a lower prevalence of folate deficiency of 11.1% among ageing rural community, ascribing to the higher folate deficiency cutoff of 9.5nmol/L as well as socioeconomic background of the population which is known to consume plant-based diets [17]. Another study done in India also found a lower prevalence of isolated folate deficiency of 12% with folate cut off value of <3ng/ml (6.8 nmol/L). This lower prevalence was explained by the study population, who were all healthy urban adults, who have better socioeconomic backgrounds, with access to various types of food, including green leafy vegetables and supplements, as well as better education on methods of food preparation, to retain folate [12].

The proportion of combined deficiencies in our study was 2.9%, lower than the 10% prevalence of previous study by Mahajan et.al, (2015) which was attributed to a high number of strict vegans (33%) in their cohort [19]. Differences in deficiency cut-offs across different countries and laboratories also contributed to variation in reported prevalence around the world, as such, a higher observed prevalence is likely when a less stringent vitamin cutoff is applied. Currently, there is lack of consensus on defining vitamin B12 and folate deficiency due to differences in measurement methods and population characteristics. However, recent studies by Ispir et al. and Kristensen et. al using the data from the national Qualimedlab EQAS showed acceptable concordance across most analytical platforms [22-23].

Our study demonstrated that Indian ethnicity was the most consistent significant factor associated with all deficiencies. This is consistent with previous studies identifying the Indian population as having the highest national prevalence of vitamin B12 and folate deficiencies, largely attributed to the widespread vegetarianism and a preference for well- cooked meal [11,18-19], which can destroy folate. Khor et al., (2006) also found a lower percentage of Indians taking dietary supplements compared to the Malays and Chinese [16]. Similar to previous studies, deficiency in both B12 and folate were largely attributable to inadequate intake while in contrary, analysis of NHANES data from 1999-2006 by Reinstaller et al., (2012) revealed that vitamin B12 deficiency in general population in US and in western population is more commonly due to the intrinsic factor deficiency and hence

malabsorption of vitamin B12 [24]. This leads to uniform guidelines for treating vitamin B12 deficiency with injectable preparations in western literature, while vitamin B12 deficiency in eastern countries is usually recommended to be treated with oral vitamin B12 [11,24].

Additionally, age group 12-19 years and 20-29 years were found to be significant predictors as well as showing the highest proportion of folate deficiency in our study, in line with UK findings reporting a 17% prevalence in adolescents (12-18 years) compared to 11% in the general population, likely due to poor dietary habits [14]. Male subjects had a higher risk of folate deficiency, consistent with findings by Hao et al. (2003) in a Chinese population [25]. This gender difference has been explained by higher rates of cigarette smoking and alcohol consumption among men. Okumura et.al reported that tobacco smoke exerts direct effects on blood folate level through the release of nitrous oxide, which inhibits methionine synthase activation and lowers endogenous B12. This, in turn, prevents the remethylation pathway and results in folate trapping in the form of 5-methyltetrahydrofolate [26]. Halsted et al. (2002) demonstrated that chronic alcoholism contributes to folate deficiency through intestinal malabsorption, decreased hepatic uptake, and increased renal excretion [27][27]. In contrast, studies by Nath et.al and Alaimo et.al found no gender differences in serum folate levels [28-29].

Another factor contributing to variations in prevalence of folate deficiency is genetic variation, which was explored in several studies previously [30-31]. The U.S Centre of Disease Control and Prevention (CDC), as reported by Cider et al. (2012) mentioned the variation in methylenetetrahydrofolate reductase (MTHFR) genes results in different individual's blood folate levels despite similar amount of folate intake [32]. The study reported that people with similar folate intake, the MTHFR TT (MTHFR C677T variant) genotype have an average about 16% lower amount of folate in their blood than those with the MTHFR CC (normal) genotype [30-31].

A statistically significant association and a high proportion of macrocytic anaemia and megaloblastic anaemia were observed in all deficiency types in our study. These findings are consistent with Mahajan et.al., (2015) who reported a prevalence of 37% macrocytosis and 23% megaloblastic anaemia deficient patients [19]. Our findings also aligned with another study, which showed that 80% and 88% of subjects who had megaloblastic anaemia had very low B12 and folate levels, respectively, compared to only 4% and 24% non-megaloblastic anaemia controls [33]. These significant associations and high proportions represent a public health concern, as these anaemic patients may require blood transfusions, an outcome potentially avoidable with earlier B12 or folate supplementation.

The strengths of our study include its time and cost-efficient retrospective cross-sectional design, utilizing data from an electronic laboratory information system (LIS). To the best of our knowledge, this is the first study to explore the prevalence of vitamin B12, folate, and combined deficiencies in the general population of Malaysia, and possibly in Southeast Asia. It may also serve as a pilot study for future research. A key limitation is potential recruitment bias, as our samples were drawn from patients and outpatients seeking medical attention at Hospital Teluk Intan, Hospital Tapah, public health clinics in Hilir Perak, Bagan Datuk, and Batang Padang districts. This may have led to an imbalance in demographic distributions (ethnicity, age, gender), health status, and comorbidities. A prospective study with a specific study population would be more likely to accurately represent the true prevalence of these deficiencies.

Conclusion

This study revealed significant racial disparities in the prevalence of serum vitamin B12, folate, and combined deficiencies among patients in a district hospital. Age and gender were also identified as risk factors for folate deficiency. These differences were likely influenced by

social determinants, which need to be further explored. Additionally, the significant association and high proportion of macrocytic and megaloblastic anaemia across all types of deficiencies raised concerns about the public health burden. Therefore, larger multicentre studies across Malaysia and Southeast Asia are recommended to establish the national prevalence of these deficiencies and identify their associated risk factors. This would, in turn, aid in developing effective screening strategies and targeted supplementation for high-risk groups, ultimately improving clinical outcomes and reducing healthcare disparities.

Acknowledgement

We would like to thank the Director General of Health Malaysia for his permission to publish this article as well as Hospital Director Hospital Teluk Intan and Head of Pathology Department for the support and assistance in performing this research.

Conflict of interest

None

Funding

None

Authors' contributions

SAII, LPC, NS and CWYS contributed to the research, background writing, literature review, preliminary writing of the manuscript, as well as data collection and analysis. IMAK, NASA, SJS, and NHS contributed to the manuscript review and facilitation of data collection and analysis.

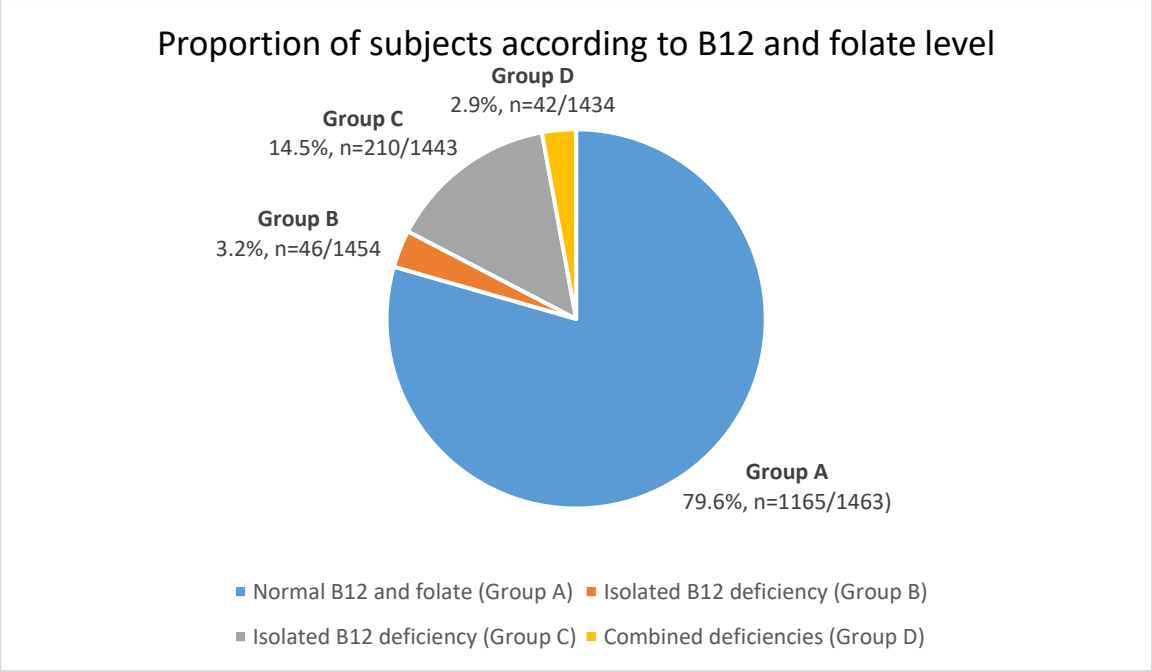

Figure 1. Pie chart showing the proportion of subjects according to B12 and folate level

Table 1. Baseline characteristics of the study population according to serum B12 and Folate level:

Characteristics	Total subject (n=1463) 100%	Group A (n=1165) 79.6%	GroupB (n=46) 3.2%	Group C (n=210) 14.5%	GroupD (n=42) 2.9%	p-value
Gender (%)						
Male	621 (42.4%)	474 (40.7%)	13 (28.3%)	98 (46.7%)	22 (52.4%)	
Female	842 (57.6%)	691 (59.3%)	33 (71.7%)	112 (53.3%)	20 (47.6%)	
Ethnicity (%)						
Malay	818 (55.9%)	692 (59.4%)	8 (17.4%)	113 (53.8%)	5 (11.9%)	
Indian	354 (24.2%)	228 (19.6%)	25 (54.3%)	70 (33.3%)	31 (73.8%)	
Chinese	247 (16.9%)	211 (18.1%)	12 (26.1%)	18 (8.6%)	6 (14.3%)	
Orang Asli	44 (3.0%)	34 (2.9%)	1 (2.2%)	9 (4.3%)	0 (0%)	
Age,year(SD)	58.1 (18.2)	59.3 (17.6)	62.0 (17.6)	51.5 (21.7)	55.0 (17.8)	<0.05[a]
Age group						
12-19, yr	34 (2.3%)	9 (0.8%)	1 (2.2%)	24 (11.4%)	0 (0%)	
20-29, yr	88 (6.0%)	60 (5.2%)	2 (4.3%)	21(10.0%)	5 (11.9%)	
30-39, yr	141 (9.6%)	109 (9.4%)	2 (4.3%)	25 (11.9%)	5 (11.9%)	
40-49, yr	191 (13.1%)	150 (12.9%)	8 (17.4%)	26 12.4%)	7 (16.7%)	
50-59, yr	240 (16.4%)	205 (17.6%)	4 (8.7%)	25 (11.9%)	6 (14.3%)	
60-69, yr	315 (21.5%)	264 (22.7%)	10 (21.7%)	35 (16.7%)	6 (14.3%)	
70-79, yr	301 (20.5%)	240 (20.6%)	14 (30.4%)	36 (17.1%)	11 (26.2%)	
>80, yr	153 (10.5%)	128 (11.0%)	5 (10.9%)	18 (8.6%)	2 (4.8%)	
Median B12 (IQR)	427 .0 (266.0,756.0)	478.0 (311.0, 835.0)	109.0 (109.0,120.7)	307.0 (183.5, 524.5)	109.0 (109.0, 120.7)	<0.05[b]
Median Folate (IQR)	16.9 (8.40,37.7)	23.6 (12.2, 41.5)	7.1 (5.0, 28.7)	5.3 (5.0, 6.2)	5.0 (5.0, 5.65)	<0.05[b]

[*] Group A = Subjects with normal B12 and folate level

Group B = Subjects with isolated B12 deficiency

Group C = Subjects with isolated folate deficiency

Group D = Subjects with combine B12 and folate deficiencies

[a] ANOVA test

[b] Kruskal Wallis-test

Table 2. Chi-square analysis of factors associated with B12, folate or combined deficiencies:

	Pearson's Chi-square	p-value	Cramer's V
Gender			
Isolated B12 deficiency	0.27	0.60[a]	-
Isolated folate deficiency	14.28	<0.05[a]	0.10
Combined deficiencies	1.69	0.19[a]	-
Ethnicity			
Isolated B12 deficiency	91.03	<0.05[a]	0.25
Isolated folate deficiency	47.05	<0.05[a]	0.18
Combined deficiencies	61.27	<0.05[a]	0.20
Age group			
Isolated B12 deficiency	7.86	0.34[b]	-
Isolated folate deficiency	14.28	<0.05[a]	0.25
Combined deficiencies	6.63	0.42[a]	-

[a] Chi-square test | [b] Fisher's Exact test

Table 3. Multinomial logistic regression analysis explaining the factors associated with deficiency status

Variable	Category	Isolated B12 deficiency			Isolated folate deficiency			Combined deficiencies		
		OR (e^B)	95% CI of OR	p-value	OR (e^B)	95% CI of OR	p-value	OR (e^B)	95% CI of OR	p-value
Gender	Female (R)									
	Male	0.94	0.84, 3.25	0.13	2.22	1.65, 2.99	<0.05	1.74	0.91, 3.32	0.09
Ethnicity	Malay (R)									
	Indian	11.83	6.36, 22.01	<0.05	2.63	1.91, 3.62	<0.05	15.88	6.09,41.42	<0.05
	Chinese	4.60	2.17, 9.74	<0.05	0.71	0.43, 1.16	0.17	4.16	1.22, 14.21	0.20
	Orang Asli	1.55	0.19, 12.29	0.67	1.14	0.50, 2.61	0.74	0.00	0.00, 0.00	0.99
Age group	12-19 years	0.57	0.07, 4.71	0.42	21.09	9.12,48.76	<0.05	0.00	0.00,0.00	0.99
	20-29 years	1.65	0.62, 4.39	0.60	3.01	1.67, 5.43	<0.05	3.42	0.96,12.18	0.50
	30-39 years	0.74	0.28, 1.91	0.30	1.83	1.06, 3.16	0.02	1.63	0.47,5.64	0.44
	40-49 years	1.44	0.67, 3.09	0.54	1.42	0.84, 2.38	0.18	1.76	0.56,5.55	0.32
	50-59 years	0.77	0.33, 1.79	0.34	0.92	0.55, 1.53	0.74	1.22	0.38, 3.96	0.73
	60-69 years (R)									
	70-79 years	1.53	0.78, 3.00	0.55	1.31	0.82, 2.09	0.25	1.80	0.64,5.07	0.26
	>80 years	0.85	0.33, 2.17	0.21	1.18	0.65, 2.14	0.56	0.72	0.14, 3.74	0.70

R= reference group for each variable measured

Table 4. Chi-square analysis of association of B12, Folate or combined deficiency with macrocytic and megaloblastic anaemia:

	Pearson's Chi-square	p-value	Cramer's V
Macrocytic anaemia[a]			
Isolated B12 deficiency	128.6	<0.05[c]	0.40
Isolated folate deficiency	45.15	<0.05[c]	0.13
Combined deficiencies	88.2	<0.05[c]	0.35
Megaloblastic anaemia[b]			
Isolated B12 deficiency	199.7	<0.05[c]	0.56
Isolated folate deficiency	86.5	<0.05[c]	0.10
Combined deficiencies	117.9	<0.05[c]	0.47

[a] Based on indices from full blood count or full blood picture

[b] Based on full blood picture morphology

[c] Fisher's Exact test

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