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Research paper

The longitudinal relationship between nutritional status and anaemia among Malaysian adolescents

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ABSTRACT

Introduction: The triple burden of malnutrition characterised by stunting and wasting, overweight/obesity, and anaemia experienced by Malaysians causes severe and long-lasting damage during the period of development and rapid growth, particularly in adolescence. This study aimed to demonstrate the trend of anaemia prevalence and to determine its longitudinal association with nutritional status and lifestyle among Malaysian adolescents.

Method: The study involved secondary data analysis from the Malaysian Health and Adolescents Longitudinal Research Team (MyHeART) study. A closed cohort secondary data analysis was performed from the dynamic cohort of 528 adolescents (male = 151; female = 377) aged 13 years attending secondary school who were followed up at 15 and 17 years. Anaemia status was determined by haemoglobin level < 12g/dL based on FBC, and iron deficiency anaemia (IDA) was determined when the Mentzer Index < 13. A generalised estimating equation (GEE) was constructed to investigate the longitudinal relationship between nutritional status and lifestyle on anaemia status over five years.

Results: The trend of anaemia prevalence increased significantly across the age group (7.9%; 95% CI: 2.3-11.1, 13.9%; 95% CI: 10.8-15.7 and 15.8%; 95% CI: 3.8-23.1) at 13, 15 and 17 years, respectively, especially among females. The trend of anaemia prevalence among females, also increased significantly across the age group (11.1%;95% CI:6.7-17.8, 15.7%;95% CI:11.4-21.3, 23.1%;95% CI:16.8-31.0). A similar trend was noted for the prevalence of IDA among those who were anaemic (66.5%; 95% CI: 40.4-85.3, 72.2%;95% CI: 54.8-85.4, 76.3%; 95% CI: 59.2-87.7). A longitudinal analysis using GEE revealed that adolescents who did not meet the Recommended Nutrient Intake (RNI) for total iron intake per day were significantly associated with anaemia (RR=1.517;95% CI: 1.012-2.275; p=0.044) and IDA (RR=1.776;95% CI: 1.225-2.57; p= 0.002).

Conclusion: The overall trend of anaemia among adolescents is in increasing trend and anaemia is prevalent among female adolescents in this study. It is crucial to understand that the current fortification strategy may need to be revisited, and robust intervention programmes are necessary and should be sex specific.

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Research in context

Evidence before this study

Anaemia among adolescence is highly prevalent in low and middle-income countries, and adolescents particularly females are prone to iron deficiency anaemia when they enter adulthood and when they become pregnant. We also searched PubMed and Google Scholar between August and September 2019, for literature on anaemia classification published, using the search terms "anaemia", "haemoglobin", "dietary intake", "iron intake", "nutritional status", "secondary school students", "Malaysia", "short stature", "BMI", "iron deficiency anaemia", "Mentzer Index" and "adolescents". Additionally, we searched for WHO technical reports on the worldwide prevalence of anaemia, the Mentzer Index as a screening tool for iron deficiency anaemia and the role of dietary intake on anaemia among adolescents. Smaller scale studies have assessed anaemia and associated micronutrient deficiencies in school children and adolescents and rural communities in Malaysia. We also found reports providing national estimates on anaemia among various age groups or iron deficiency anaemia, which is the focus of the national anaemia prevention programme.

Added value of this study

The Malaysian Health and Adolescents Longitudinal Research Team (MyHeART) study is the first large adolescent cohort study to undertake a detailed assessment of anaemia based on haemoglobin testing using the gold-standard cyanmethemoglobin method and estimation of iron deficiency anaemia in a nationally representative sample of children and adolescents aged 13-17 years old. This study also conducted a complete anthropometric assessment and dietary history in a large sample of healthy adolescents that includes both genders. This enabled us to investigate an adolescent cohort over five years over three measurement points. Mentzer Index was used as a screening tool to differentiate iron deficiency anaemia and beta thalassemia trait. There is an increasing prevalence of low intake of total iron intake (below the recommended nutrient intake-RNI) among the adolescents over the period noted from our study. Our study also reports that the prevalence of anaemia among adolescents is increasing in trend and the prevalence of iron-deficiency anaemia is most prevalent among females. Iron deficiency anaemia accounts for more than two third of anaemia in female adolescents. There is also a longitudinal association found between total iron intake and the risk of developing anaemia and iron deficiency anaemia in adolescents. None of these findings has been reported previously by any national population-based study in Malaysia or Asia.

Implications of the available evidence

Iron deficiency anaemia is the most common form of anaemia in female adolescents, and there is a need to focus on anaemia prevention efforts like iron and folic acid supplementation programme in the country for the targeted population. At the same time, the increasing prevalence of anaemia highlights the need for the national programme to address these deficiencies. More research is needed to understand anaemia of other causes. The MyHeART study serves as a baseline for future studies or surveys to evaluate the national nutrition and anaemia prevention programme among adolescents in the country.

Introduction

Adolescents in developing countries are commonly affected by micronutrient deficiency, resulting in anaemia. In developing countries, almost one-quarter of adolescents are anaemic [1], but in the South-East Asia region, the prevalence estimates of adolescent anaemia range from 27% to 55% [2]. The vulnerability of adolescents to anaemia is mainly due to the increased biological demands for micronutrients like iron and folic acid associated with rapid physical growth. In contrast, females remain vulnerable to anaemia due to menstrual blood loss [3]. Males regain adequate nutrient stores rapidly at the end of adolescence, while females continue to be anaemic or become more anaemic due to increased micronutrient requirements from menstrual loss and teenage pregnancy [4]. World Health Organization (WHO) regional estimates generated for preschool-age children, pregnant and non-pregnant women indicate that the highest proportion of individuals affected are in Africa (47.5–67.6%), while the greatest number affected are in South-East Asia where 315 million (95% CI: 291–340) individuals in these three population groups are affected [3].

A population-based Anaemia Screening by Awaluddin et al. in 2017 showed that anaemia's national prevalence was 24.16% (95% CI: 23.16-25.19), which can be estimated further to nearly 5 million people. The prevalence of anaemia among females aged 15 to 19 years was significantly higher compared to males of the same age (N=469) (34.14%; 95% CI: 30.71-37.75 vs. 9.62%; 95% CI: 7.74-11.89)(5). A study by Yusoff et al. among secondary school students in Kelantan revealed that anaemia is a significant public health problem in Malaysia with an overall prevalence of 59.6% [6].

In 2012, the World Health Assembly Resolution 65.6 endorsed a Comprehensive Implementation Plan on Maternal, Infant and Young Child Nutrition (CIP), with six Global Nutrition Targets for 2025. The second target is a 50% reduction of anaemia in women of reproductive age (15-49 years)(7) Anaemia is interlinked with other Global Nutrition Targets; stunting, low birth weight, exclusive breastfeeding and wasting. Targeted actions are required to reach the anaemia target by 2025, and the 2nd and 3rd Sustainable Development Goals (SDG) of reducing all forms of malnutrition and ensuring healthy lives for all ages by 2025. This requires nutrition-specific interventions for preventing and controlling anaemia throughout the life cycle.

There was limited data to explain the anaemia situation among Malaysian adolescents as previous studies were more focused on children and pregnant women. Efforts to understand the anaemia status and lifestyle factors towards this public health problem in adolescents could help develop early strategies to prevent adverse health-related outcomes. Hence, this study sought to present the trend of anaemia prevalence and determine its longitudinal association with nutritional status and lifestyle among Malaysian adolescents, therefore assist appropriate and timely action to be taken to achieve the target for anaemia as mentioned by WHO and SDG.

Methods

The STROBE statement for the cohort was adhered to in reporting this study.

Study design and area

This was a closed cohort secondary data analysis of the Malaysian Health and Adolescents Longitudinal Research Team (MyHeART) study. The MyHeART study is a prospective and dynamic cohort study of a representative sample of Malaysian adolescents residing in Peninsular Malaysia. This study applied a two-stage clustered sampling method. Further details of the study design and sampling procedure have been described in its published protocol [8].

Study population

The sampling frame consisted of all secondary school students within the Federal Territory of Kuala Lumpur (as a metropolitan), Selangor (Central region), and Perak (Northern region). The participants were recruited at 13 years for baseline (W_0) assessment in 2012. The first follow-up (W_1) assessment was performed in 2014 (age: 15 years old) and the second follow-up (W_2) assessment took place in 2016 (age: 17 years old). Identification numbers were used to track the adolescents in the closed cohort.

The inclusion criteria were that students must be able to write and read in Malaysia's national language (Malay). Students in other types of schools like boarding, religious, and vernacular schools were excluded from the study because the majority belong to a single ethnic group. A written information sheet about the study and consent forms were distributed to the participants and their parents or guardians to be completed and indicate their willingness to participate.

In total, 15 schools participated in 2012 (baseline) with a total of 1333 students eligible for recruitment. However, nine students were excluded because they did not have a Full Blood Count (FBC) result, while 60 were excluded for not completing the seven-day diet history booklet, and 11 were excluded for not taking part in the anthropometry assessment. Finally, a total of 1255 students were recruited for baseline in 2012 and follow-up for five years. Subsequently, some students were lost during the first and second follow-ups: 428 (34.1 %) and 244 (31.6 %) in the first and second follow-ups, respectively. One participant was excluded due to implausible energy intake during W_2 data collection. Thus, the sample size for this closed cohort for analysis was only 528. The sampling flow for this study is explained in Figure 1. In this study, 13-year-old adolescents were referred to as early adolescence, 15-year-old adolescents: middle adolescence and 17-year-old adolescents: late adolescence.

Sociodemographic measures

The sociodemographic data on sex, ethnicity, place of residency, parent's socioeconomic status, education level and occupation were collected using validated questionnaires for both the parents and students [9,10].

Anaemia status

Anaemia status among adolescents is considered the dependent variable. Fasting blood samples (11cc) were drawn from the antecubital vein under aseptic technique and distributed into a plain, EDTA (Ethylenediaminetetraacetic Acid) and fluoride test tubes which were sent to the laboratory for FBC analysis. Anaemia status was determined based on the WHO guidelines on the definition of haemoglobin (Hb) cut-off point(11) and further classified by its severity into mild, moderate and severe. Anaemia is defined as Hb below 12.0 g/dL for children aged 12-15 years and females aged 15 years and above, and below 13.0 g/dL for males aged 15 years old and above. This cut-off value for haemoglobin is unadjusted for altitude since it is not crucial to do adjustment based on the geography of the study site.

Iron deficiency anaemia and thalassemia are some of the most common causes of microcytic hypochromic anaemia. The inability to differentiate between the two conditions based on blood film and the expensive cost of testing such as electrophoresis and Hb analysis led to some researchers using various indices to differentiate between these two conditions. In this study, the researcher applied the Mentzer Index (MI), which is the ratio of Mean Corpuscular Volume (MCV) / Red Blood Cell (RBC) count. When the quotient is less than 13, this finding is most likely seen in thalassemia, and if the quotient is greater than 13, this is most likely seen in IDA [12,13]. The results of children who were anaemic were immediately issued through the class teacher to their parents for immediate further action at the nearest clinic for the required treatment.

Dietary assessment

Seven-day diet history was chosen for this study to assess the habitual food and beverage intakes because this method has produced more valid estimates of energy intake than other methods. It is also most preferred for epidemiological studies [14] since adolescents can recall better than adults [15]. The participants were interviewed by trained dietitians who asked open-ended questions to obtain information on daily food and drinks that students consumed for breakfast, mid-morning snack, lunch, afternoon tea, dinner and supper over the seven days. The Nutrient Composition of Malaysian Food (4th edition) [16] via the Nutritionist Pro database (Axxya Systems) was used to analyse the total intake and obtain an average intake of seven days of total energy, protein and iron. The Nutritionist ProTM Extraction Tool (Axxya Systems) was used to extract the calculated average daily intake of energy and nutrient intakes. Data cleaning was subsequently performed, which involved checking the consistency, errors, and correctness of the data entered and implausible energy intake.

Anthropometric measurements

Each child's height and weight were measured to determine their anthropometric indices by trained personnel. The Height-for-Age Z-score(8) and Body Mass Index-for-age Z-score (BAZ) were calculated using Anthro-plus open-access software (Anthro-Plus v1.0.4, WHO, Geneva, Switzerland) to assess underweight, obesity and short stature status, respectively, as indicators of poor nutritional status. HAZ and BAZ were compared with WHO Growth Reference Standards. Children were classified as underweight, obese or short stature respectively when their Z-scores fell two or more standard deviations below the mean value.

Ethics

The original MyHeART study's ethical approval was obtained from the Medical Ethics Committee, University Malaya Medical Centre (MEC Ref.No: 896.34). This was a voluntary study and consent forms were signed by the participants and parents/guardians who agreed to participate.

Statistical analysis

Descriptive and inferential statistical analyses were performed using IBM Statistical Package for Social Science (SPSS) version 22(17). The significance level was set at $p < 0.05$. Since this study employed a multi-stage sampling design as described before, therefore an extra variable called 'weightage' was created for every respondent. The longitudinal analysis was performed using data from baseline (W_0), first follow-up (W_1) and second follow-up (W_2). A generalised estimating equation was used to assess the trend of nutritional status (BMI for age Z-score, energy, protein, and iron intake) using data from baseline at 13 years old (W_0), first follow-up at 15 years old (W_1) and second follow-up at 17 years old (W_2). A generalised estimating equation (GEE) was also used to evaluate the longitudinal effect of energy, protein, iron

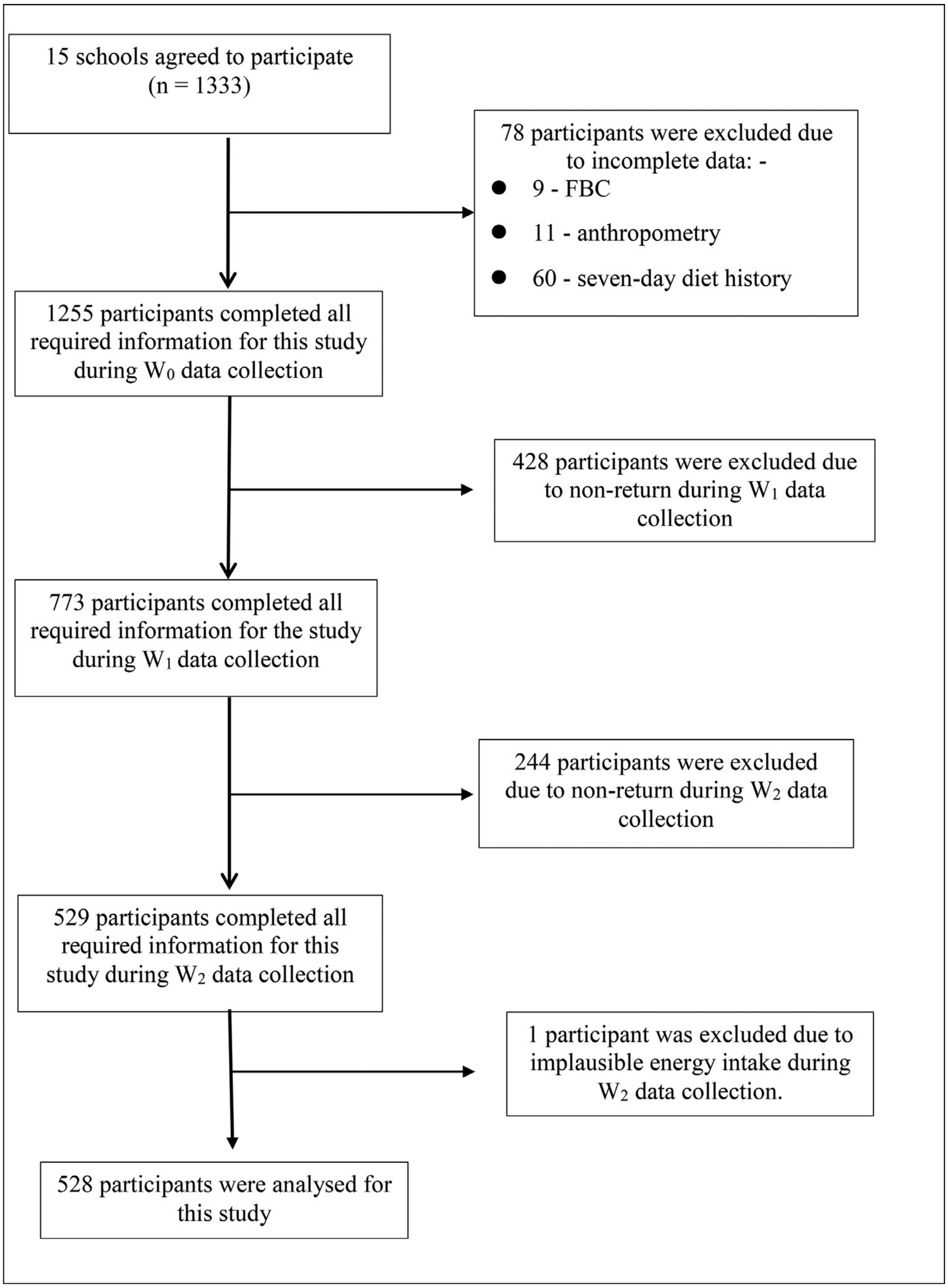

Figure 1. Sampling flow for this study.

intake and BMI for age Z-score on the changes in the trend of anaemia over the 5 years. Variables that had a p-value < 0.25 in the univariate analysis were selected to be included in the analyses to prevent important variables from being missed. The interaction effect between the predictors and time exposure has been tested to see whether the is an effect between these two. The distribution of the outcomes (anaemia) was set as Poisson and the link function as log. The working correlation structure in this GEE model was independent as there was no missing data after multiple imputations and the time of follow-up for each participant was equal. Multiple imputation was the method opted for this study due to the nature of the missing data, which is not due to missing completely at random (MCAR). Only data from participants with complete data for diet history, full blood count and anthropometry measurement for all three-time points were included in this analysis.

Results

A total of 1255 participants' complete data were used for analysis. Of this number, 773 participants returned for this study during the first follow-up at 15 years old (W1) and subsequently 529 participants turned up when they were 17 years old (W2). The follow-up rate for the total sample was 42.2% within five years based on Figure 1 that shows the flow of the study. Like most adolescent cohort studies, this follow-up rate ranged between 40% to 51% [16,17]. Since this was a voluntary study, some of the parents refused to take part in the following years.

Sociodemographic characteristics of study participants at baseline

The descriptive sociodemographic of the adolescents recruited in this study are presented in Table 1. Most of the participants

Table 1
Sociodemographic characteristics of respondents at baseline (2012).

Variable of Interest	Frequency (N)	Percentage (%)
Region		
Kuala Lumpur	209	39.6
Selangor	197	37.3
Perak	122	23.1
Gender		
Male	151	28.6
Female	377	71.4
Locality		
Urban	335	63.5
Rural	193	36.5
Ethnicity		
Malay	357	67.6
Chinese	52	9.9
Indian	105	19.9
Others	14	2.7
Socioeconomic status (SES)		
Low	258	48.8
Middle	230	43.6
High	40	7.5
Mother's Occupation		
Homemaker/Unemployed	263	49.9
Working Fulltime	175	33.2
Working Part-time	89	16.8
Studying Fulltime	4	0.7
Mother's Education		
No Schooling	7	1.3
Primary	58	11.0
Secondary	372	70.4
Tertiary	77	14.7
Others	14	2.6
Father's Occupation		
Unemployed	40	7.6
Working Fulltime	421	79.8
Working Part-time	67	12.6
Father's Education		
No Schooling	8	1.6
Primary	41	7.8
Secondary	408	77.2
Tertiary	61	11.5
Others	10	1.9

were female Malay urban residents with low socioeconomic status. The majority of the participants' mothers are homemakers whereas the fathers are employed. Both parents received their education until the secondary level.

The trend in anaemia prevalence and dietary intake

The prevalence of dietary intake of adolescents who did not meet RNI is shown in Table 2. The overall prevalence of inadequate iron intake (unmet RNI) among adolescence increased significantly more than two-folds from 13 years old to 15 years old. This trend continued to increase till they were 17 years old. On contrary, the prevalence of inadequate total energy and protein intake however reduced significantly when they were 15 years old.

Table 3 showed that the overall prevalence of anaemia almost doubled at the age of 15 years old compared to the baseline. A further increase followed this in the prevalence of anaemia towards the end of the second follow-up. Upon comparison between gender, it was noted that anaemia was higher among females, and the trend was increasing in nature. The prevalence of anaemia among females when they were 17 years old was noted to be almost double the finding when they were 13 years old.

The MI which was used as a screening tool to differentiate between IDA and Beta Thalassemia Trait (BTT), showed that more than half of those diagnosed with anaemia were found to be IDA. The trend of IDA was noted to increase over the five years. The analysis showed that IDA was most prevalent among anaemic females compared to males. The prevalence of IDA significantly increased from 15 years old to 17 years old among female adolescents.

Longitudinal Analysis

Table 4 shows the results of the longitudinal association between exposures and anaemia among adolescents. The GEE analysis showed that unmet RNI for daily total iron intake was found to cause a higher risk of anaemia with the RR=1.517 (95% CI 1.012,2.275, $p<0.05$). It means that adolescents who did not meet their RNI for daily total iron intake have a 51.7% increase in the risk of developing anaemia compared to those whose daily RNI for iron is met.

The longitudinal association between exposures and IDA among adolescents is shown in Table 5. The GEE analysis showed that those who did not meet the RNI for iron were at significantly higher risk of anaemia with the RR=1.776 (95% CI:1.225,2.575, $p<0.01$)

Discussion

The trend in anaemia prevalence and dietary intake

This is the first study to analyse a longitudinal cohort to investigate adolescent anaemia in Malaysia. It reveals that the overall prevalence of anaemia among adolescents aged 17 years old in 2016 was 15.8%. Overall, the trend of anaemia for females of this study was at an upward trend similar to a study among Japanese adolescents [18]. This is lower when compared to the prevalence rate reported in the National Health and Morbidity Survey (NHMS) 2015 where the prevalence of anaemia among adolescent 15-19 years (N=469) was reported as 21.4% (95% CI: 19.3-23.7(5, 19). We could not compare the findings of the anaemia prevalence in this study with the global estimation based on data available in the Micronutrients Database of the WHO (Vitamin and Mineral Nutrition Information System – VMNIS) because adolescents, the elderly and men were excluded from the database [11]. The findings of anaemia prevalence among females 23.1% (95%CI: 16.8-31.0) from our study are similar to the findings from the Global Nutrition Report 2018 (24.9% of women aged 15 to 49 years) which also mentioned that no progress had been made towards achieving the target of reducing anaemia among women of reproductive age. NHMS 2017, which focused on adolescent health and nutrition, did not include blood parameters that could have detected anaemia and other risk factors for non-communicable diseases [19]. This finding is, however, lower compared to the finding of NHMS 2015 where the prevalence of anaemia among females 15 to 19 years was significantly higher (34.14%; 95% CI: 30.71-37.75) [5,19]. However, the finding of our study on the prevalence of anaemia among females at 17 years old is comparable to the findings of the prevalence of anaemia in women of reproductive age group based Global Nutrition Report 2019 which is 21.4% [7]. This makes Malaysia one of the 41 countries that struggles with three forms of malnutrition.

The overall trend of low iron intake (did not meet RNI) among adolescence increased over time with a significant peak in those aged 15 and 17 years. Rapid physical growth during adolescence, menarche and resulting blood loss may also reduce the iron levels of women of reproductive age and lead to anaemia, especially in older girls. A study in the United States showed that adolescent girls who have more than three years of menstrual history had three times the risk of anaemia than youth with less than three years of menstruation [20]. The NHMS 2017 adolescent survey reported that 52% of Malaysian adolescents are getting the recommended composition of nutrients from carbohydrates, 15% in protein and 33% in fat [21]. We need to understand that the RNI for

Table 2
Dietary intake of adolescent who did not meet RNI for energy, protein and iron by age of follow-up (n=528).

Dietary Intake	W_0 (2012) 13 years			W_1 (2014) 15 years			W_2 (2016) 17 years		
(Did not meet RNI)	Prevalence %	SE	95%CI	Prevalence %	SE	95%CI	Prevalence%	SE	95%CI
Total Energy Intake	74.9	3.0	(68.5, 80.3)	42.6*	3.6	(35.7,49.7)	54.8	3.7	(47.6,61.8) $p<0.0001$
Protein Intake	24.1	3.0	(18.8, 30.4)	8.9*	1.6	(6.2, 12.6)	13.6	2.1	(10.0,18.3) $p<0.0001$
Total Iron Intake	26.6	3.0	(21.1, 32.9)	63.9*	3.7	(56.4,70.8)	77.8**	3.1	(71.3,83.2) $p<0.0001$

* Statistically significant between baseline (W_0) and first follow-up (W_1).
** Statistically significant between first follow-up (W_1) and second follow-up (W_2).

Table 3
Anaemia prevalence from 2012 to 2016 by sociodemographic factors, Mentzer Index, and nutritional status (N=528).

	2012		2014		2016		
	n (%)	95% CI	n (%)	95%CI	n (%)	95%CI	p-value
Overall Individual	50(8.0)	5.0 -12.3	87(13.9)*	10.0 -19.0	92(15.8)	11.3 -21.7	
Gender							<0.0001
Male (N=151)	7(2.6)	1.0-6.3	13(10.8)	4.4-22.9	4(3.8)	7.0-17.0	
Female (N= 377)	43(11.1)	6.7-17.8	74(15.7)*	11.4-21.3	88(23.1)**	16.8-31.0	
Mentzer Index							
≥13(IDA)	40(80.8)	40.4-85.3	63(72.7)	54.8-85.4	70(76.3)	59.2-87.7	
<13(BTT)	10(19.2)	14.7-59.6	24(27.3)	54.8-85.4	22(23.7)	12.3-40.8	0.028
Mentzer Index							
IDA							
-Male	3(38.2)	8.6-80.3	11(84.7)	57.8-95.7	0	-	
-Female	30(70.4)	39.5-89.7	50(67.7)	47.3-83.1	74(83.8)**	73.5-90.6	<0.0001
BTT							
-Male	4(61.8)	19.7-91.4	2(15.3)	4.3-42.2	4(100.0)	100.0	
-Female	13(29.6)	10.3-60.5	24(32.3)	16.9-52.7	14 (16.2)	9.4-26.5	
Residence							
Urban	20(57.9)	36.9-76.4	34(50.6)	34.3-66.8	43(65.0)	50.2-77.3	
Rural	30(42.1)	23.6-63.1	53(49.4)	33.2-65.7	49(35.0)	22.7-49.8	<0.0001
Ethnicity							
Malay	37(50.4)	28.3-72.3	68(61.2)	41.2-78.0	69(48.8)	32.0-65.8	
Chinese	3(9.0)	2.8-25.4	1(1.7)	0.2-11.3	1(1.5)	0.2-10.1	
Indian	8(38.2)	16.3-66.1	12(32.8)	16.2-55.3	12(42.6)	24.8-62.5	<0.0001
Others	2(2.5)	0.6-10.0	6(4.2)	1.8-9.6	10(7.2)	3.5-14.2	
Socioeconomic status (SES)							
Low	29(57.1)	33.7-77.6	47(63.0)	48.1-75.8	54(65.3)	48.4-79.1	
Middle	19(38.4)	18.7-62.8	35(32.3)	20.8-46.7	34(31.4)	18.3-48.3	<0.0001
High	2(4.6)	0.9-20.1	5(4.5)	1.6-12.4	4(3.3)	1.0-10.4	
Height for Age, Z-score							
Short stature	0	-	5(7.1)	2.6-18.0	10(14.2)	5.0-34.2	0.006
Normal Height for Age	50(100.0)	100.0	82(92.9)	82.0-97.4	82(85.8)	65.8-95.0	
BMI							
Underweight	0	-	6(6.4)	2.6-15.0	8(12.6)	4.0-33.6	
Normal	34(76.7)	59.8-87.9	59(66.9)	49.5-80.6	61(64.3)	45.7-79.4	<0.0001
Overweight/Obesity	16(23.3)	12.1-402	22(26.7)	14.1-44.8	23(23.1)	11.7-40.4	
Total calorie intake/day							
Meet RNI	0	-	1(0.6)	0.1-4.1	19(14.5)	8.1-24.7	
Did not Meet RNI	50(100.0)	100.0	86(99.4)	95.9-99.9	73(85.5)*	75.3-91.9	<0.0001
Protein intake/day							
Meet RNI	32(71.7)	53.1-85.1	77(85.7)	63.4-95.4	75(74.4)	53.6-88.0	<0.0001
Did not Meet RNI	18(28.3)	14.9-46.9	10(14.3)	4.6-36.6	17(25.6)	12.0-46.4	
Iron Intake/day							
Meet RNI	33(76.1)	12.4-41.1	13(24.3)	11.7-43.7	7(13.3)	4.3-34.3	<0.0001
Did not Meet RNI	17(23.9)	58.9-87.6	74(75.7)*	56.3-88.3	85(86.7)	65.7-95.7	

* Statistically significant between baseline (W_0) and first follow-up (W_1).
** Statistically significant between first follow-up (W1) and second follow-up (W_2).

protein, iron and energy differ between gender and age as they grow older [22] unfortunately his was not considered in NHMS 2017 and the Malaysian Adult Nutrition Survey (MANS).

The second peak of IDA seen in adolescents is primarily due to rapid growth often combined with poor dietary intake of iron. These contributors may be compounded by menstrual blood loss in adolescent females [23]. The MI is one of the RBC indices and formulas that have been based on the highest sensitivity and specificity in discriminating BTT from IDA. This formula helps classify patients with hypochromic microcytic anaemia into two essential categories, so specific tests can be requested for Hb electrophoresis or serum iron to save time and reduce diagnostic expenses [24,25].

Longitudinal association between nutrition status and anaemia/IDA

GEE was implemented while controlling for gender, residency, SES, maternal education, paternal occupation, physical fitness score (PFS) and HAZ. The current study did not analyse the longitudinal association based on gender because most of the adolescents

Table 4
Results of GEE to assess the longitudinal association between nutrition status and anaemia among adolescents.

Variables	B	SE	RR (95%CI)	p-value
BMI for age Z-score				
Underweight	-0.692	0.5907	0.501 (0.157,1.593)	0.241
Normal			1.000	
Overweight/Obese	-0.098	0.221	0.906 (0.587,1.398)	0.656
Total energy intake/day (kcal/d)				
Did not meet RNI	0.204	0.199	1.226 (0.830,1.811)	0.306
Met RNI			1.000	
Total protein intake/day (g/d)				
Did not meet RNI	-0.198	0.22	0.821 (0.530,1.270)	0.375
Met RNI			1.000	
Total iron intake/day (mg/d)				
Did not meet RNI	0.417	0.207	1.517 (1.012,2.275)	0.044*
Met RNI			1.000	

* Statistically significant at $p < 0.05$.
B: Beta Coefficient; SE: Standard Error, RR: Relative Risk, CI: Confidence Interval.
Quasi-Likelihood Under Independence Model Criterion (QIC)=44419.84, Corrected Quasi-Likelihood Under Independence Model Criterion (QICC)=39846.06.
Adjusted for 'time', gender, residency, SES, maternal education, paternal occupation, PFS and HAZ.

Table 5
Results of GEE to assess the longitudinal association between nutrition status and iron deficiency anaemia (IDA) among adolescents.

Variables	B	SE	RR (95%CI)	p-value
BMI for age Z-score				
Underweight	-0.373	0.638	0.688 (0.197,2.404)	0.558
Normal			1.000	
Overweight/Obese	-0.064	0.221	0.938 (0.609,1.445)	0.772
Total energy intake/day (kcal/d)				
Did not meet RNI	0.096	0.181	1.100 (0.772,1.568)	0.597
Met RNI			1.000	
Total protein intake/day (g/d)				
Did not meet RNI	-0.234	0.256	0.792 (0.47,1.308)	0.362
Met RNI			1.000	
Total iron intake/day (mg/d)				
Did not meet RNI	0.574	0.189	1.776 (1.225,2.575)	0.002*
Met RNI			1.00	

* Statistically significant at $p < 0.05$.
B: Beta Coefficient; SE: Standard Error, RR: Relative Risk, CI: Confidence Interval.
Quasi-Likelihood Under Independence Model Criterion (QIC)=3172.091, Corrected Quasi-Likelihood Under Independence Model Criterion (QICC)=28266.536.
Adjusted for 'time', gender, ethnicity, residency, SES, PFS and short stature.

who were found to be anaemic are females. However, this study found a longitudinal association between total iron intake/day with anaemia and IDA among adolescents. Several cross-sectional studies found that dietary iron intake is significantly associated with anaemia [26]. To the best of the researcher's knowledge, no cohort study has investigated the longitudinal relationship of nutritional status and anaemia among adolescents from early until late adolescence to date worldwide. Often, these cohort studies were analysed cross-sectionally either looking at single nutrients like the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study [27], adolescent preconception health in the aDolescent and prEconception health peRspectiVe of Adult Non-communicable diseases (DERVAN) study [28] or dietary pattern [29].

Based on current findings, this warrants an emphasis on creating a conducive school environment that promotes healthy eating with adequate nutrients to promote a healthier body and prevent malnutrition.

Strengths and Limitations

The longitudinal design remains one of the strengths of our study. It is the first adolescent cohort study conducted in Malaysia and among very few in Asia with a comprehensive blood profile, anthropometric assessment and dietary history and a large sample of healthy adolescents that includes both genders. It enabled us to investigate an adolescent cohort over five years over three measurement points, especially when enrolment at the secondary level in public schools was 89.27% in 2019 [30]. This study's major strength is the robustness of the tools used, whereby all anthropometric information is based on objectively measured data. The Mentzer Index's inclusion as a screening tool for IDA is another advantage of our study, which has been noted as an important factor to consider in other studies [31].

Although an appropriate dietary assessment method is used, under-reporting and over-reporting of dietary intake are inevitable, but this has been overcome when the analysis was conducted. This limitation may reduce the strength of any observed association. The researcher also recognises that recall bias is a natural limitation of dietary research. However, this diet histories would be a reasonable approach for low and middle-income countries (LMIC) studies, and they have shown that the memory processing of adolescents is better than that of adults and the tool is suitable for this age group [15].

Conclusion

This study provided insights into the dietary intake in early adolescence, the trend of anaemia prevalence, longitudinal association between dietary intake and anaemia, and introduced a new screening method to differentiate between IDA and BTT. A healthy food environment and providing optimum nutrition are crucial for the adolescents. Further intervention studies for this group are important as they will have an impact on the nutrition policy development of the country.

Data sharing statement

Participant data is not publicly available. Access to datasets can be directed to the corresponding author.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Author Contributions

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