UNIVERSITI PUTRA MALAYSIA

ASSOCIATIONS BETWEEN VITAMIN D-RELATED FACTORS (DIETARY, SUPPLEMENT USAGE AND SUN EXPOSURE LEVEL) AND HAEMOGLOBIN CONCENTRATION AMONG FEMALE STUDENTS IN UNIVERSITI PUTRA MALAYSIA (UPM)

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BY

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A project submitted as partial fulfilment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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ABSTRACT

ASSOCIATIONS BETWEEN VITAMIN D-RELATED FACTORS (DIETARY, SUPPLEMENT USAGE AND SUN EXPOSURE LEVEL) AND HAEMOGLOBIN CONCENTRATION AMONG FEMALE STUDENTS IN UNIVERSITI PUTRA MALAYSIA (UPM)

Nurul Izzah Abdullah Binti Mohd Noradlin

Vitamin D deficiency has been shown to lead to reduced iron absorption due to the upregulation of hepcidin hormone resulting in anaemia. However, it remains unclear whether dietary, vitamin D supplement intakes and sun exposure level (SEL) are associated with haemoglobin (Hb) concentration. This cross-sectional study was carried out to investigate the associations between those factors and Hb concentration among 155 female students aged 19-26 years (22.40 ± 1.34). A set of questionnaire was used to determine the socio-demographic characteristics and anthropometric measurement were performed. Dietary vitamin D intake and supplement usage were determined using the Food Frequency and Lifestyle Questionnaire whilst SEL was determined using a SEL questionnaire and reported as Sun Exposure Index (SEI). Hb concentration was assessed using HemoCue© Hb 201+ Analyzer and anaemia was defined using WHO cut off points of <12 g/dl. Pearson and spearman correlations were used to determine the associations between vitamin D-related factors and Hb concentration where appropriate with level of significance set at $p<0.05$. Anaemia prevalence was 38.7%, with a mean ± SD Hb concentration of 12.10 ± 1.38 g/dl. Mean ± SD dietary and supplemental vitamin D intakes were 8.10 ± 5.08 µg/day and 13.56 ± 6.38 µg/day, respectively. SEL/week was 2.08 ± 1.11 hours with BSA fraction of 0.30 ± 0.22 and SEI of 2.50 ± 1.46. The study found associations between dietary vitamin D intake (r=0.28, p=0.01) and vitamin D supplement use (r=0.40, p=0.019) with Hb concentration. However, no significant association was observed between the SEL, BSA fraction and SEI with Hb concentration, which may be due to the SEL and SEI were considered low in overall. This study demonstrates that increased in dietary and supplements vitamin D consumption led to improved Hb concentration but warrants further investigations. Efforts to promote the importance of vitamin D should emphasise, including adequate dietary vitamin D intake, vitamin D supplementation and the need for exposure to sunlight that could improve health and Hb status of the female students.

ABSTRAK

PERKAITAN ANTARA FAKTOR YANG BERKAITAN DENGAN VITAMIN D (DIET, PENGGUNAAN SUPPLEMEN DAN TAHAP PENDEDAHAN MATAHARI) DAN KEPEKATAN HAEMOGLOBIN DALAM KALANGAN PELAJAR WANITA DI UNIVERSITI PUTRA MALAYSIA (UPM)

Nurul Izzah Abdullah Binti Mohd Noradlin

Kekurangan vitamin D telah terbukti menyebabkan penyerapan zat besi berkurang disebabkan oleh peningkatan hormon hepcidin yang mengakibatkan anemia. Walau bagaimanapun, masih belum jelas sama ada pengambilan makanan, pengambilan supplemen vitamin D dan tahap pendedahan cahaya matahari dikaitkan dengan kepekatan hemoglobin (Hb). Kajian keratan rentas ini dilakukan untuk mengkaji hubungan antara faktor tersebut dan kepekatan Hb di kalangan 155 pelajar perempuan berumur 19-26 tahun (22.40 ± 1.34). Satu set soal selidik digunakan untuk menentukan ciri-ciri sosio-demografi dan pengukuran antropometri dilakukan. Pengambilan makanan dan supplemen vitamin D ditentukan dengan menggunakan soal selidik Kekerapan Makanan dan Soal Selidik Gaya Hidup sementara tahap pendedahan cahaya matahari (SEL) ditentukan menggunakan soal selidik SEL dan dilaporkan sebagai Indeks Pendedahan Matahari (SEI). Kepekatan Hb dinilai menggunakan HemoCue © Hb 201+ Analyzer dan anemia ditentukan menggunakan titik pemotongan WHO <12 g / dl. Korelasi pearson dan spearman digunakan untuk menentukan hubungan antara faktor yang berkaitan dengan vitamin D dan kepekatan Hb di mana sesuai dengan tahap kepentingan yang ditetapkan pada p <0,05. Prevalensi anemia adalah 38.7%, dengan kepekatan ± SD Hb 12.10 ± 1.38 g / dl. Purata ± SD diet dan pengambilan vitamin D tambahan masing-masing 8.10 ± 5.08 µg / hari dan 13.56 ± 6.38 µg / hari. SEL / minggu adalah 2.08 ± 1.11 jam dengan pecahan BSA 0.30 ± 0.22 dan SEI 2.50 ± 1.46. Kajian mendapati hubungan antara pengambilan vitamin D diet (r = 0.28, p = 0.01) dan penggunaan suplemen vitamin D (r = 0.40, p = 0.019) dengan kepekatan Hb. Namun, tidak ada hubungan yang signifikan antara SEL, pecahan BSA dan SEI dengan kepekatan Hb, yang mungkin disebabkan oleh fakta bahawa SEL dan SEI dianggap rendah secara keseluruhan. Kajian ini menunjukkan bahawa peningkatan pengambilan makanan dan pengambilan vitamin D menyebabkan peningkatan kepekatan Hb tetapi memerlukan penyelidikan lebih lanjut. Usaha untuk mempromosikan kepentingan vitamin D harus dititikberatkan, termasuk pengambilan makanan vitamin D yang mencukupi, suplemen vitamin D dan keperluan untuk pendedahan kepada cahaya matahari yang dapat meningkatkan kesihatan dan status Hb pelajar wanita.

CHAPTER 1

INTRODUCTION

Anaemia is a common blood disorder, which can be defined as a low blood haemoglobin (Hb) concentration than normal, which is insufficient to meet an individual physiological need (WHO, 2015). This blood disorder is a global disease and has significant adverse health impacts on people in low-, middle-, and high-income countries (WHO, 2015). At the population level, Hb concentration is the most accurate indication of anaemia, which is defined as Hb levels of less than 12 g/dL in females and less than 13 g/dL in males (WHO, 2015). Pregnant women and children were more likely to be affected with anaemia and global health observatory's anaemia database reported prevalence of anaemia was 29.4% among reproductive-aged women with the children having the highest prevalence (42.6%) and non-pregnant women having the lowest (29.0%) (WHO, 2015).

Globally, the highest rates of anaemia in non-pregnant women were found in Africa (47.5%) and South-East Asia (35.7%), whilst the lowest incidence were observed in the United States (US) with 17.8% prevalence (McLean et al., 2009). In Malaysia, 1 in 5 Malaysians adults were anaemic, which corresponds to approximately 4.6 million of the population (NHMS, 2019). Based on observation from the National Health and Morbidity Survey (NHMS) in 2019, it was reported the overall country prevalence of anaemia was 21.3% and nearly 15.9% of women in reproductive age were diagnosed with mild anaemia. Thus, present study was focus on university student as university student is in women reproductive age and prevalence of anaemia among them is quite high.

Several studies have been carried out in Malaysia, demonstrated a wide range of anaemia prevalence between 28.3% and 48.5%. These studies were carried out in pregnant women (35%) (Haniff et al., 2007), school children (48.5%) (Al-Mekhlafi et al., 2008), adolescent girl (28.3%) (Chang et al., 2009), adults (24.6%) (Awaluddin et al., 2017), and elderly, (35.3%) (Yusof et al., 2018). In another study carried out in Sabah, it was demonstrated that there were 32% of female adolescent aged between 12 to 19 years old were anaemic and nearly 88% of those young women were reported to be anaemic due to iron deficiency (ID) (Milman, 2015).

Anaemia has many consequences, but the most noticeable health implications of severe anaemia are increased risk of mother and infant death, and maternal morbidity (Khaskheli et al., 2016). It is associated with a variety of physiological effects, including poor tissue oxygen supply, weakness, tiredness, decreased productivity due to decreased labour capability, cognitive impairment, and higher susceptibility to infection (Haas & Brownlie IV, 2001). Numerous variables, including rapid growth, hormonal changes, and the onset of menstruation in females, have been identified as major contributors to anaemia throughout adolescence (Balci et al., 2012) Malnutrition and bad eating habits is the factors that have been demonstrated to lead to anaemia in both genders in students (Campillo et al., 2004). However, evidence also showed that lack in dietary intake of iron, folate could contributed to anaemia (Chai et al., 2019). Another study carried out by Lopez et al. (2016) found that iron is required in large amounts on a daily basis for red blood cell formation and cellular metabolism; however, because dietary intake is insufficient, alternative sources of iron are required for iron homoeostasis.

There was recently emerging evidence of the utilization of vitamin D, which has been shown to act as iron absorption enhancer affecting the iron bioavailability through the suppression of hepcidin, the main iron regulator. An investigation showed that vitamin D concentrations are oppositely associated with hepcidin concentrations which insufficient vitamin D may cause the upregulation of hepcidin which would inhibits iron uptake from the gut (Basutkar et al., 2018). This may promote the decline of Hb levels and increase the rate of anaemia (Carvalho et al., 2011). Vitamin D insufficiency has been found to increase the likelihood of iron deficiency anaemia in healthy female children and young adult due to iron degradation and also been shown to have association with anaemia the elderly, those with severe kidney disease (CKD), and those suffering from heart failure in previous research (Smith & Tangpricha, 2015). According to recent study, maintaining an adequate vitamin D level may be important for avoiding anaemia, particularly in inflammatory disorders (Smith & Tangpricha, 2015). Vitamin D is a well-known necessary nutrient involved in a variety of immunological activities in addition to skeletal and muscular growth (Prentice, 2008).

Numerous countries around the world reported a significant frequency of vitamin D insufficiency, ranging from 19.3% to 51.4% in including US (24%), Canada (37%), Europe (40%) (Amrein et al., 2020), Turkish (41.3%), Moroccan (36.5%) with the greatest incidence was shown in Surinam South Asian (51.4%) and the lowest was 19.3% in sub-Saharan African (Edwards et al., 2014). Based on National Health and Nutrition Examination Survey (NHANES), the incident of vitamin D deficiency (25(OH)D <50 nmol/l) was 28·9% and vitamin D insufficiency ($50 \leq$ 25(OH)D $<$

75nmol/l) was 41·4% adults in the US during 2007 to 2014 (Liu et al., 2018). The high prevalence of vitamin D deficiency in Malaysia was found in the study carried out by Moy and Bulgiba (2011). It was sought to determine the prevalence of vitamin D insufficiency (50 nmol/L) in an existing Malay cohort aged 35 years and above in Kuala Lumpur. Only one-third of individuals had appropriate vitamin D levels (50 nmol/L), while roughly 41% and 87% of males and females, respectively, had insufficient 25-hydroxyvitamin D levels (50 nmol/L) (Moy & Bulgiba, 2011).

To maintain optimal vitamin D level, sufficient dietary intake of vitamin D is essential (Forrest & Stuhldreher, 2011). There was a study to determine the association between dietary vitamin D intake and anaemia carried out by Michalski et al. (2017) among women aged 18 to 40 years in Vietnam (n=4961) and it was demonstrated that vitamin D intakes were significantly but weekly associated with anaemia and Hb concentration (r= 0.03, p= 0.02) (Michalski et al., 2017). A semi-quantitative food frequency questionnaire (FFQ) containing a list of 107 common foods and beverages consumed in Vietnam was used to assess dietary vitamin D intake in a study carried out among the participants (Michalski et al., 2017). However, a study carried out to examine the effect of providing 200 mg/d calcium and 25 g/d vitamin D in a fortified food product on iron status in 152 young adults undergoing military training, discovered a contradict finding where no differences in iron status between the treatment and placebo groups following calcium and vitamin D fortified food consumption (Hennigar et al., 2016).

Vitamin D supplementation was the other determinants of vitamin D parameters which could have association with anaemia. Vitamin D supplementation's influence on Hb concentration was shown by a recent study using randomised controlled trials (RCTs) (Arabi et al., 2020). The study has shown that the Hb of participants was not reduced and that vitamin D3 supplementation in adults between 20 and 500 000 IU had no significant impact on levels of Hb Arabi et al. (2020). A study carried out by Braithwaite et al. (2019) performed another study in the UK to evaluate the effectiveness of prenatal vitamin D3 supplementation in hepcidin suppression or inflammation in 93 women over the age of 18. It was found a connection between the amounts of serum 25(OH)D and hepcidin ($p= 0,006$) as well as ferritin ($p<0,001$) (Braithwaite et al., 2019). Hepcidin is known as iron suppressor where it inhibits ferroportin, the transmembrane protein responsible for iron transfer, and hence during low iron status, hepcidin will be suppress to optimise iron absorption from food and the release of iron from stored iron (Braithwaite et al., 2019). On the contrary, Masoud et al. (2018) carried out a study conducted to assess supplement vitamin D treatment could alter iron status among 125 Arab young adults. The study found that a six month supplementation vitamin D intake of 1000 IU/day had a substantial effect in decreasing serum iron levels ($p= 0.002$) (Masoud et al., 2018). On the contrary, study carried out by Madar et al. (2016) in Norway among 251 adults aged 18 to 50 years found that supplementation of vitamin D3 (25µg) had no significant association on Hb ($r= 0.04$), serum ferritin ($r= 0.06$), serum iron ($r= -0.05$) or transferrin saturation ($r= -0.004$).

While vitamin D can be derived from dietary sources and supplement, the primary source of vitamin D is from the activation of dehydrocholesterol through the exposure of skin to ultraviolet B (UVB) radiation (Green et al., 2008). Hence, factors such as season, latitude, clothing, skin colour and time of day could influence vitamin D status (Green et al., 2008). The high prevalence of low serum vitamin D concentrations in all age groups, including in regions with high sun exposure, is a global issue (Hamilton et al., 2014). Young women have risk factor of insufficiency vitamin D due to malnutrition, less outdoor activity and frequent use of sun protection (Yoo & Cho, 2015). In a study carried out by Pourzand et al. (1999) demonstrate that UVA radiation causes an immediate increase in the amount of iron that can be used by the body by degrading the ferritin molecule. Ferritin synthesis is stimulated at both the transcriptional and translational stages as a result of the iron-regulatory protein 1 downregulation, leading to a higher amount of the iron storage protein (Pourzand et al., 1999).

However, most study used serum 25(OH)D levels indicators to determine the association of vitamin D with anaemia. A cross sectional study carried out among children and adolescents found serum 25(OH)D levels was associated with increased risk of anaemia (OR= 1.81, CI: 1.13- 2.88), iron deficiency anaemia (OR= 2.26, CI: 1.20-4.24) and iron deficiency (OR= 1.94, CI: 1.27-2.97) in both children and adolescents (Lee et al., 2015). Another cross sectional study carried out among community-dwelling adults over the aged of 17 years old found low 25(OH)D levels were associated with increased risk of anaemia (Monlezun et al., 2015). Another study carried out by Doudin et al. (2018) in 5066 German adolescents aged 11 to 17 years found that serum 25(OH)D was inversely associated with Hb ($r = -0.04$, $p = 0.003$) but no significant association were found in the either iron ($r = -0.02$, $p = 0.128$), mean corpuscular Hb ($r = 0.01$, $p = 0.690$), or ferritin ($r = 0.02$, $p = 0.172$). A cross-sectional study carried out among patients with End-Stage Renal Disease (ESRD) found 25(OH)D3 deficiency was significantly associated with anaemia ($\beta = 0.263$, $p < 0.001$) (Kim et al., 2016). In addition, they found patients who have less than 10 ng/mL of 25(OH)D3 serum level had a significantly higher risk for developing anaemia (Hb level <10 g/dL) compared to patients who have more than 10 ng/mL of 25(OH)D3 ($p = 0.036$) (Kim et al., 2016) .

However, serum 25(OH)D has been found to have association with anaemia with inflammation as reported by Smith and Tangpricha (2015). The mechanism underlying this association involves the reduction of pro-inflammatory cytokines by vitamin D as well as the direct inhibition of hepcidin mRNA transcription (Smith & Tangpricha, 2015). Another pathway contributing to this inflammation is through reduced erythropoiesis and serum 25(OH)D has been shown to support erythropoiesis by increasing burst-forming unit-erythroid proliferation thus low serum 25(OH)D will suppressed erythropoiesis and causing anaemia with inflammation (Nemeth & Ganz, 2014; Aucella et al., 2003).

Previous studies were focused on serum 25(OH)D as the primary indication of vitamin D status, rather than particular vitamin D factors such as dietary and sun exposure level (SEL) as indicators of deficiency in vitamin D. Recent developments in our understanding of the relationship between serum 25(OH)D and anaemia show that maintaining adequate vitamin D status may be crucial in preventing anaemia, particularly in inflammatory diseases. Thus, other indicators were needed to further develop the better understanding between this association. Moreover, the magnitude of problem connecting vitamin D and anaemia is emerging, but there is lack of Hb studies especially among women of reproductive age in Malaysia. Apart from that, prior research mostly assessed iron status using a variety of markers that were not specific to Hb concentration. Besides, the prevalence of anaemia in Malaysia is relatively high. Although the highest prevalence of anaemia was in children, a female student is the earliest age of bearing. Thus, this present study aimed to determine the association between vitamin D-related factors (diet, supplement usage and SEL) with Hb concentration among female students in Universiti Putra Malaysia (UPM).

1.1 Research Questions

The present research aims to address the following questions:

1) What was the Hb concentration of female students in UPM?
2) How much was the vitamin D intake from diet, supplement usage and SEL of female students in UPM?
3) What was the associations between diet, supplement usage, SEL and Hb concentration among female students in UPM?

1.2 Objectives

1.2.1 General Objective

To determine the associations between vitamin D-related factors (dietary, supplement usage and SEL) and Hb concentration among female students in Universiti Putra Malaysia (UPM).

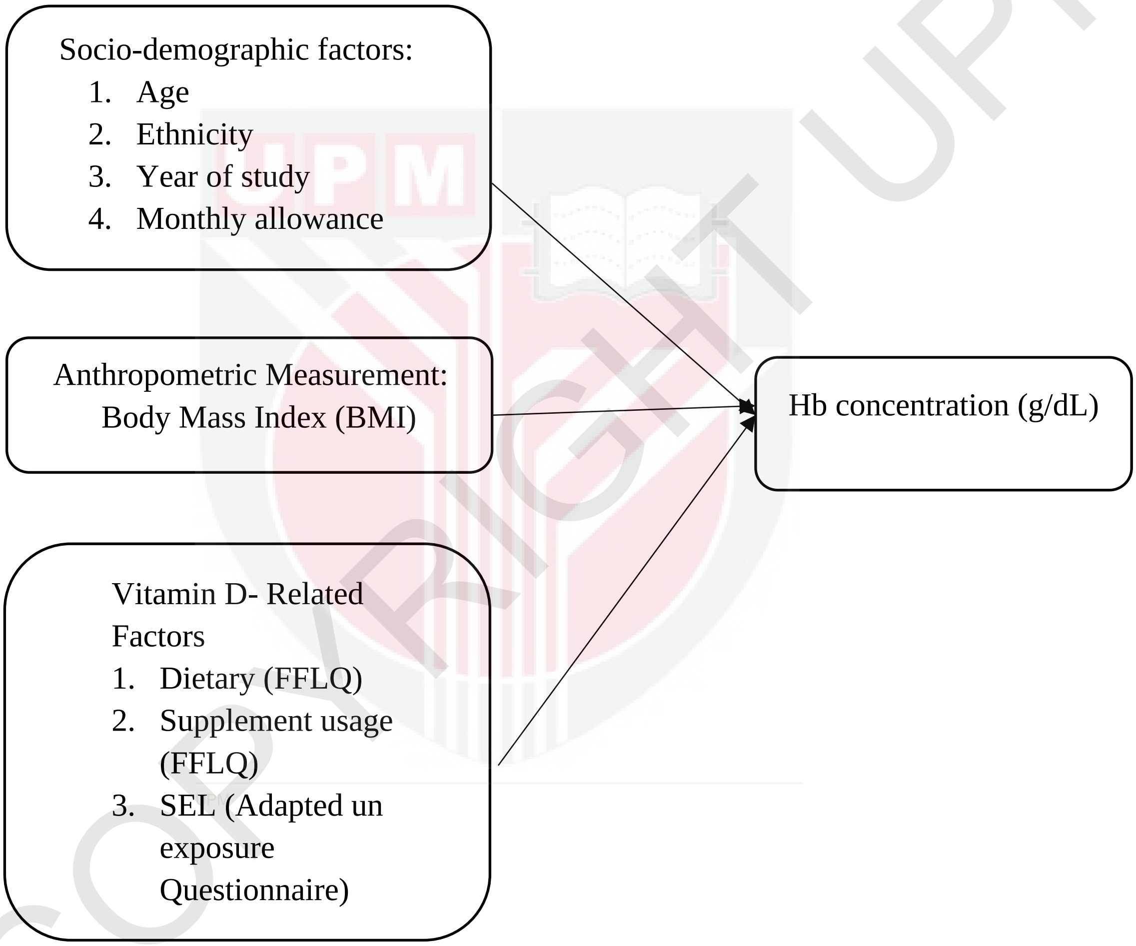
1.2.2 Specific Objectives

1. To determine the sociodemographic characteristics of female students in UPM
2. To determine the anthropometric measurements of female students UPM
3. To determine vitamin D-related factors (dietary, supplement usage and SEL) of female students in UPM
4. To determine the Hb concentration of female students in UPM
5. To determine the associations between dietary vitamin D intake, vitamin D supplement usage, SEL and Hb concentration among female students in UPM

1.3 Hypotheses

There were associations between vitamin D-related factors (dietary, supplement usage and SEL) and Hb concentration among female students in Universiti Putra Malaysia (UPM).

1.4 Research Framework



CHAPTER 2

LITERATURE REVIEW

2.1 Iron

2.1.1 Iron Status

Iron is a transition metal and among the most abundant nutrient on the earth which exist in two distinct form which are ferrous (Fe^{2+}) and ferric (Fe^{3+}) (Oliveira et al., 2014). In aqueous solutions, ferric hydroxide ($Fe(OH)^3$) is formed spontaneously when Fe2+ is oxidised by molecular oxygen to Fe^{3+} (Oliveira et al., 2014). Therefore, the maximum solubility of iron in the oxidative environment, such as extracellular fluids, is controlled by the equilibrium constant of $Fe(OH)^3$ (Oliveira et al., 2014). The overall solubility of Fe^{3+} at physiological pH is very low, while the solubility of Fe^{2+} is much greater (Oliveira et al., 2014). Iron need to be in Fe^{2+} form or bonded to haem which is a type of the protein, in order to be stored in the body (Ems & Huecker, 2019). According to MOH (2017), there are four major classes of iron exist in the human body. Iron containing haem proteins such as Hb, myoglobin, and cytochrome are important for transporting oxygen, electron, and storage whilst iron sulphur enzymes including flavoproteins and heme-flavoproteins are actively involved in energy metabolism. Iron mainly exists as haem (approximal 2 g of iron in men and 1.5 g of iron for women) in erythrocytes while exist in a lesser measure as myoglobin in muscle cells and in the storage composition as ferritin and hemosiderin (Ems & Huecker, 2019).

Iron is involved in various cellular functions in human body. In human body, iron is delivered to tissue through circulation of transferrin which function as a transporter capturing iron discharge into the plasma (Dev & Babitt, 2017). Then, iron is transported to mitochondria for the production of haem or iron sulphur clusters, followed by the storage of excess iron in ferritin molecules (Dev & Babitt, 2017). A review by Abbaspour et al. (2014) stated that iron absorption occurs mostly in the duodenum and upper jejunum of small intestines. Iron is then transferred across the duodenal mucosa into the blood by transferrin and then transported to the cell or bone marrow for the production of red blood cells. In terms of iron excretion, there is no physiological mechanism but the loss of iron is only balanced by intestinal immersion where most of the iron was recycled when old red blood cells were withdrawn from circulation and destroyed by splenic macrophages then returned to the storage for re-used (Duck & Connor, 2016).

Wallace (2016) stated that it is important to maintain the optimal level of iron in circulation as iron plays important roles in cells and tissues functioning as well as immune responses. Too little iron can cause anaemia and other chronic inflammation whilst iron overload can result in tissue damage and infection, including fibrosis of the liver, endocrine failure and cardiomyopathy. Hence, to maintain iron balance, the loss of iron must be replaced through the consumption of iron by the diet.

2.1.2 Anaemia

Iron deficiency anaemia is among the most common health concerns in the world, despite its abundance on the earth (Soleimani & Abbaszadeh, 2011). The WHO has reported that 1.62 billion people worldwide are anaemic (McLean et al., 2009). Iron deficiency anaemia accounts for 75% of all anaemia in the third world and affects 30% of the population (Soleimani & Abbaszadeh, 2011). According to WHO (2015), anaemia affects around 1.62 billion people worldwide, or approximate to 24.8% of the population. The greatest incidence of anaemia was observed in preschool-aged children (47.4%) whilst men have the lowest prevalence of anaemia (12.7%) (McLean et al., 2009). In terms of population group that are vulnerable towards anaemia, WHO (2015) stated that nearly 468.4 million of non-pregnant women was found to have the greatest number of individuals affected by anaemia. In the context of Malaysian adult's population, the prevalence of anaemia was 24.16% corresponding to 5 million people (Awaluddin et al., 2017). In terms of specific age groups, elderly was found to have the highest prevalence of anaemia (35.27%) whilst in term of gender, females (34.50%) had a significantly higher prevalence of anaemia than males (14.4%). Indians (30.84%) have been found to have the highest prevalence of anaemia compared to other ethnicities including Malays and Chinese (Awaluddin et al., 2017).

Iron deficiency (ID) develop in stages and can be measure by various biochemical indices (Abbaspour et al., 2014). Iron status can be determined by measuring Hb concentrations, serum ferritin levels, serum iron and transferrin concentrations, and total iron-binding capacity concentrations (TIBC) (McLean et al., 2009). Measuring Hb concentration is the most valid method to determine the prevalence of anaemia in a population (WHO, 2015). It was suggested that Hb concentration is an alternative gold standard to assess iron status among anaemic people (Daru et al., 2017). Distributions of normal Hb differ with age, sex, residential elevation (altitude), smoking status, and physiological status such as pregnancy

(WHO, 2015). Hb concentration will be reduced below the normal levels when the red blood cell has insufficient iron (WHO, 2015).

Iron is important in carrying oxygen in red blood cells to provide enough energy to the body. The most common cause of iron deficiency anaemia in females of reproductive age is blood iron loss due to heavy menstruation or pregnancy (Al Hassand, 2015). Anaemia also may be affected by a poor diet or certain intestinal disorders that impair the body's ability to absorb iron (Al Hassand, 2015). Poor diet can be cause by lack dietary iron intake. Dietary iron is divided into two forms which are haem iron and non-haem iron (Young et al., 2018). Haem iron has greater efficacy of iron absorption (15-40% of intake) as compared to non-haem iron (1-15% of intake) (Briguglio et al., 2020). This is due it is easily pass through the membranes into the blood vessels with the help of a specific haem transporters (Young et al., 2018). Haem iron mainly comes from animal sources such as meat, fish and poultry where it accounts for approximately 40% of the total iron whilst non-haem iron is from plant foods and iron-fortified food products (National Institutes of Health, 2019 & Briguglio et al., 2020). Foods with non-haem iron are usually much less absorbed compared to haem-iron, but the requirement of non-haem iron is important for vegetarians and vegans who rely solely on non-haem iron (Young et al., 2018). Iron can also be found in certain foods added with iron known as fortified food products (National Institutes of Health, 2019).

Iron bioavailability is defined as the amount of iron that can be ingested from food and utilised for regular bodily processes (Hurrell & Egli, 2010). According to MOH (2017), the quantity of iron absorbed from food is mostly controlled by the amount of iron stored in the body and the bioavailability of iron in various diets and age groups. Meats and foods that are rich in ascorbic acid were found to have highest iron bioavailability (15%-35%) whilst cereals, tubers, and legumes-based diets with little or no meat or ascorbic acid were found to have lowest iron bioavailability (2%-20%) (Hurrell & Egli, 2010). The consumption of enhancers including ascorbic acid may improve the bioavailability of non-haem iron (Cepeda-Lopez, Melse-Boonstra, Zimmermann, & Herter-Aeberli, 2015).

2.1.3 The Inhibitor and Enhancer of Iron Absorption

The quantity of iron absorption from a food or meal is determined by physiological factors such as body iron status and dietary inhibitors and enhancers (Beshara et al., 2003). Vitamin C, meat, chicken, fish, and alcohol are the primary dietary enhancers of iron absorption, whereas tannins (found in tea and coffee), calcium and dairy products, polyphenols, phytate, animal proteins (milk and eggs), and other micronutrients such as zinc and copper are the primary inhibitors (Hurrell & Egli, 2010). The literature on iron bioavailability has highlighted in several studies. Phytate as the main inhibitor of iron absorption and the amount inhibited absorption by 18% ($p<0.001$), 64% ($p<0.001$) and 82% ($p<0.001$) at 2 mg, 25 mg and 250 mg respectively (Beshara et al., 2003; (Hallberg et al., 1989). Calcium has been found to suppress both non-haem and haem iron inhibits, unlike other inhibitors which only impact the absorption of non-haem iron (Hallberg et al., 1993). Animal proteins including milk, cheese and egg, have been proven to inhibit iron absorption (Cook & Monsen, 1976). In humans, casein and whey, two main bovine milk protein fractions, as well as egg white, have been shown to prevent iron absorption (Hurrell et al., 1989).

Vitamin C, often known as ascorbic acid, is a water-soluble vitamin and effective non-haem iron enhancer (Beck, 2015). Due to its capacity to decrease ferric to iron ferrous, vitamin c is more strong than other organic acids to absorb iron. (Teucher et al., 2004). Cepeda-Lopez et al., (2015) carried out a study in 62 women with normal weight (n=24), overweight (n=19), and obese (n=19) to assess absorption of iron from a meal with and without ascorbic acid. This study discovered that iron absorption from a meal without ascorbic acid was lower in normal weight (19%), overweight (12.2%), and obese (13.6%) people, while iron absorption from a meal with ascorbic acid was higher in normal weight (29.5%), overweight (14.9%), and obese (18.4%). The median percentage in iron uptake for the comparison between a meal with or without ascorbic acid was 56% in normal weight ($p<0.001$) and 28% in both overweight and obese ($p<0.006$), which reported that there was a significant increase in iron uptake from a meal with ascorbic acid in women with normal weight ($p<0.004$). Other organic acids such as citric acid, alcohol and fermented foods also could assist non-haem iron absorption. Vitamin A and carotenoids have been found in some studies to enhance non-haem iron absorption, but not in all (García-Casal et al., 1998; Layrisse et al., 2000).

Another factor that was found to act as an enhancer in iron absorption is vitamin D. Vitamin D can increase iron availability as it may promote erythropoiesis by inhibiting the expression of pro-inflammatory cytokines and hepcidin (Smith & Tangpricha, 2019). Hepcidin is a hormone involved in the regulation of systemic iron concentration in the body and then maximised the release of iron from the stores, thus increase iron absorption (Smith & Tangpricha, 2019). Through these potential mechanisms of action, vitamin D may therefore improve anaemia, in particular, anaemia of inflammation (Elizabeta Nemeth & Ganz, 2014). Iron can be sequestrated inside the cells of the reticuloendothelial system and was not accessible for erythropoiesis under chronic inflammatory circumstances which ultimately can lead to anaemia (Andrews, 2004).

2.2 Vitamin D

2.2.1 Biology of Vitamin D

Vitamin D is an essential nutrient which performs a dual role as a precursor hormone nutrient and fat soluble vitamin in the human body (Malczewska-Lenczowska et al., 2018). In general, vitamin D exists in refers to two forms which is soluble in fat, ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Certain invertebrates and fungi develop vitamin D2 whilst vitamin D3 is produced in vertebrate tissue, both after exposure to ultraviolet (UV) radiation (O'Mahony et al., 2011). Ingested vitamin D2 and endogenously formed D3 are converted to 1,25-dihydroxyvitamin D (1,25(OH)2D) (calcitriol) in the human body which is the only biologically active form of vitamin D (O'Mahony et al., 2011).

Vitamin D status can be determined using 1,25-dihydroxyvitamin D 1,25(OH)2D and 25-hydroxyvitamin D 25(OH)D (Shafinaz & Moy, 2016). However, circulating 25(OH)D is regarded a superior measure for vitamin D status since it has a lengthy circulation half-life of 14 to 20 days (Holick & Chen, 2008). In contrast, 1,25(OH)2D is less used due to its short half-life which approximately 15 hours. Besides, the level of 1,25(OH)2D usually does not decrease until there is a severe vitamin D deficiency (Cranney et al., 2007). Vitamin D, as 25(OH)D, is an important risk factor for vitamin D insufficiency. It is synthesized in the liver and is a measure of vitamin D availability through skin synthesis and nutrition (Holick, 1994).

2.2.2 Sources of Vitamin D

Vitamin D is naturally occurring in animal diets. It is present in trace amount and highly varied in butter, cream, egg yolk and liver. Fish liver oils are the finest dietary sources. The consumption of vitamin D-enriched foods, such as milk, cereals and orange juice, can enhance dietary vitamin D. Several food sources of vitamin D are given in Table 2.1.

In Malaysia, vitamin D recommended intakes (RNI) have been improved from 5 µg/day (RNI 2005) to 15 µg/day for population aged 1–70 years, and RNI are defined as the average daily dietary intake level which is enough to fulfil the nutritional demands of 97.5% of each demographic group (MOH, 2017). Inadequacy of vitamin D was characterised as a circulating marker for levels of <50 nmol/L and < 30 nmol/L of 25-hydroxyvitamin 25(OH)D, by the U.S. Institute of Medicine (IOM, 2011). However, Institute of Medicine et al. (2011) defined deficiency, insufficiency and sufficiency of vitamin d as 25(OH)D < 50, 51–74 and > 75 nmol/L, respectively (Institute of Medicine et al., 2011).

Malaysia by comparison, has a higher vitamin D level than other countries owing to its proximity to the equator. Despite this, research involving a subset of the population have revealed widespread vitamin D insufficiency. This is a unforeseen finding considering Malaysia's tropical climate, which receives an average of 7–8 hours of strong sunshine daily, significantly exceeding the 6–8 minutes required for optimal vitamin D synthesis (Al-Sadat et al., 2016).

Table 2.1 Food sources of vitamin D

Food	Vitamin D µg/100g
Poultry, Meat, Fish	
Fish, salmon, pink	10.9
Fish, mackerel, cooked	7.3
Fish, sardines, cooked	4.8
Egg, whole	2.0
Beef, liver	1.2
Fish, catfish, farmed	0.2
Beef, Meat	0.1
Chicken, Meat	0.1
Lamb, meat	0.1
Dairy	
Milk, cow fortified, low fat	1.3
Yogurt, fortified, low fat	1.2
Cheese, cheddar	1.0
Vegetables	
Mushroom, oyster	0.7
Potatoes, mashed	0.3

2.2.3 Factors Affecting Vitamin D

Vitamin D is obtained from two primary sources. Most adults, especially in the sunny regions do not have to obtain vitamin D from food sources. They can rely on natural exposure of sunlight to maintain adequate vitamin D nutrition.

2.2.3.1 Sun Exposure

Vitamin D is primarily obtained by sun exposure. Since the sun is one of the best sources of vitamin D, it is sometimes referred to as "the sunshine vitamin." (Nair & Maseeh, 2012). Sunlight is the most prevalent source of UV radiation, and it emits three types of UV rays. UVA radiation increases the amount of free accessible iron immediately by degrading the iron storage protein ferritin, which increases the quantity of the iron storage protein. However, up to 50% of the world's population does not get enough sun, and 40% of Americans are vitamin D deficient (Haq et al., 2016; Forrest & Stuhldreher, 2011). Unfortunately, prolonged exposure to sunlight can destroy vitamin D precursors in the skin, preventing them from being converted to active vitamin D (Ministry of Health Malaysia, 2017). Darker-skinned people require more prolonged sunlight exposure than light-skinned people. This is due to the dark pigment skin which acts as sun protection, but will cause reduced vitamin D synthesis. This was inline was in line with study by Al-Sadat et al. (2010) found that Indian young adults with darker skin of displayed the lowest average serum vitamin D concentrations compared with their Malay and Chinese counterparts (Al-Sadat et al., 2016). Besides, latitude, season and time of day, heavy clouds, smoke, or pollution all act as a barrier to the sun's UV radiation and may affect vitamin D synthesis (Ministry of Health Malaysia, 2017).

According to the Korean Meteorological Administration's 2008–2009 statistics, the high incidence of vitamin D insufficiency in summer may be linked to the limited length of sunshine between late June and July, due to the rainy season and thick cloud cover at that time of year (Ah Lee et al., 2008). Recent research has established that vitamin D deficiency is widespread in tropical nations such as Vietnam (Ho-Pham et al., 2010), Malaysia and Indonesia (Green et al., 2008). However, there is a lack of study that determines the association of sun exposure and anaemia in Malaysia. Aside from sunlight, vitamin D can also be obtained from dietary sources.

2.2.3.2 Dietary Vitamin D

Vitamin D is high in food sources such as fatty fish, egg yolk, and fortified dairy, and grain products. A study carried out by Jungert et al., (2014) found that consumption of fish (β=0.235; $p<0.001$) and fat intake (β=0.283; $p<0.0001$) were higher and positive determinants of dietary vitamin D intake in different sex, age, vitamin D status, body mass index, or household income among 235 elderly people living in German. There are few studies relates between vitamin d and iron. A study carried out by Toxqui et al. (2014) to determine the effect of iron and vitamin d enriched-skimmed milk on bone remodelling as low iron state is the factor of increased bone resorption in 165 iron deficient women, found that NTx (bone resorption marker) was lower in the iron and vitamin D group ($p<0.005$) at week 8, but significantly increase in the iron and vitamin D group ($p<0.001$) throughout intervention compared to the group without any supplements. The study also reported that consumption of dairy product could reduce bone turnover and increases serum 25(OH)D concentration thus nearly maintaining vitamin D status.

The findings of a study conducted by Hall et al. (2010) which examined the effects of sun exposure, dietary vitamin D intake, and skin pigmentation on serum 25(OH)D in 72 young adults at the University of California, Davis, revealed that there was a positive association between vitamin D intake and serum 25(OH)D ($r= 0.27$, $p= 0.02$), but that additional dietary vitamin D was required to achieve and maintain the serum 25(OH)D at molecular level.

2.2.3.3 Supplement Vitamin D

In pilot human trials suggest that vitamin D dietary supplementation may be helpful at reducing hepcidin mRNA expression and reducing serum hepcidin concentrations (Smith & Tangpricha, 2015). Few trials have been conducted to determine the effect of vitamin D supplementation on anaemia. Lin et al. (2004)

demonstrated that treatment with the active form of vitamin D, calcitriol, was beneficial in alleviating anaemia associated with chronic renal disease in patients receiving haemodialysis. In a study of patients with myelodysplastic syndromes, Mellibovsky et al. (1998) demonstrated that treatment with calcitriol resulted in increases in haematologic markers including Hb (Mellibovsky et al., 1998).

The present findings suggest that vitamin D supplementation may have an inhibitory effect on iron indices in healthy adolescents with suboptimal but not severely deficient 25(OH)D levels, implying that the causative association between the two micronutrients may be conditional on the severity of the shortage, the type of iron deficiency and other metabolic disorders known to have an effect on hematologic function (Masoud et al., 2018). To establish the validity of the current findings, well-designed, randomised trials are necessary (Masoud et al., 2018). Despite the lack of statistically significant improvements in plasma ferritin levels, this study demonstrated that 38g of extra vitamin D combined with fortified iron cereal improved Hb and haematocrit levels among women with low iron storage. These findings may have therapeutic implications for re-establishing iron stores in iron deficient populations at the level of healthcare (Salma & Mushtaq, 2019).

Nevertheless, in a small randomised, blinded controlled intervention, Indians ethnicity aged between 15 to 60 years with simultaneous vitamin D insufficiency received vitamin D3 intramuscularly, but the vitamin D supplementation had no effect on Hb concentrations (Sooragonda et al., 2015). An additional randomised trial in Norway for ethnic minorities with a double-blind control of placebo aged 18–50 years (Madar et al., 2016). For 16 weeks, participants were randomly assigned to receive daily oral supplementation with 10 g vitamin D3, 25 g vitamin D3, or a placebo

(Madar et al., 2016). There were no statistically significant differences in s-ferritin, Hb, s-iron, or transferrin saturation between the groups (Madar et al., 2016).

By late pregnancy, the vitamin D3 group's blood 25(OH)D level had increased by 17 nmol/L, while the placebo group's had decreased by 11 nmol/L; hepcidin, ferritin, and inflammatory markers were decreased, but no treatment group differences were seen. In late pregnancy, positive correlations between 25OHD and hepcidin and 25OHD and ferritin were seen in the placebo group but not in the treatment group (group 25OHD interaction, p= 0.02). Supplementation with vitamin D3 had no effect on hepcidin, ferritin, or the inflammatory state.

Study by Braithwaite et al., (2019) found that by late pregnancy, the vitamin D3 group had increased 17 nmol/L serum 25(OH)D, whilst the placebo group's had declined by 11 nmol/L, meanwhile hepcidin, ferritin and inflammatory biomarkers were reduced, but no changes observed among intervention group. Positive correlations between 25(OH)D and hepcidin, as well as between 25(OH)D and ferritin, were seen in late pregnancy among the placebo group, but were not observed in the treatment group. Vitamin D3 supplementation showed no impact on hepcidin, ferritin or inflammatory state, suggesting that vitamin D3 has no adjunctive utility in decreasing prenatal iron insufficiency rates. According to current recommendations, vitamin D daily dietary requirements should be increased. Radlovic et al. (2012) suggested 400IU, 600IU and 800 IU of supplement intake should be safely covering the optimal body requirements for 0-18 years, 19-70 years and for persons aged over 70 years respectively.

2.2.4 Deficiency of Vitamin D

Inadequate vitamin D status has been found to be related with a number of chronic health problems and illnesses, including type 2 diabetes, high blood pressure, and inflammatory ailments such as rheumatoid arthritis (Hossein-nezhad & Holick, 2013). Insufficient vitamin D intake is also associated with deficiency linked to rickets, osteoporosis and osteomalacia, as well as poor recovery of falls (Rosen, 2011). A large number of researches have examined the association between vitamin D and a variety of health outcomes; however, fewer studies have looked specifically at young adults (Zhang & Naughton, 2010). The insufficiency of vitamin D is increasing due to reduced consumption of fortified vitamin D meals, increased use of sunblock along with decreased exposure to sunlight. However, the lack of vitamin D is frequently caused by insufficient calcium intake and results in bone deterioration or osteoporosis (Heaney, 2008).

Deficiency in vitamin D causes impaired synthesis in the calcium, phosphate and bone (Damaso et al., 2017). Reduced vitamin D level may potentially reduce the absorption of intestinal calcium, thus raising level of parathyroid hormone level (PTH), which in turn induces 25(OH)D-1α-hydroxylase kidney synthesis, the renal enzyme that transforms 25(OH)D into 1,25(OH)2D (Damaso et al., 2017). The increase in PTH also increases osteoclastic activity which leads to bone loss and eventually to osteoporosis (Damaso et al., 2017). The lack in vitamin D has also been linked to muscle fatigue and increased risk of falling in adults and seniors, and metabolic disorder and resistance to insulin (Michael, Holick & Chen, 2008). Vitamin D is linked closely to skeletal health, and its lack may leads to childhood rickets and adult osteomalacia and osteoporosis (Liu et al., 2018). Accumulated data, however, indicates that vitamin D functions well for the optimum role of extra skeletal bodies and tissues in the human body (Liu et al., 2018). Vitamin d deficiency was related to increased cardiovascular risk (Liu et al., 2018). Higher levels of vitamin D are attributed to lower prevalence of cancer and lower deaths due to cancer (Liu et al., 2018).

2.3 Associations between Vitamin D-Related Factors and Hb Concentration

Vitamin D could increase Hb concentration by suppression of hepcidin, thus vitamin D has been one of determining factors towards. Anaemia is defined as low blood Hb concentration and a condition where there are insufficient red blood cells to meet the physiological needs of humans (WHO, 2011). Table 2.2 summarises studies investigated the association between dietary vitamin D and Hb concentration. From the studies, most of them found there was positive correlation between vitamin D and anaemia.

Table 2.2: Previous studies on the association between vitamin D related factors and Hb concentration

Study	**Study Population**	**Vitamin D level**	**Prevalence of anaemia / id /Ida/Mean Hb**	**Association between vitamin D and anaemia**
Basutkar et al. 2018 India	Pregnant Women, n=101	16.5±5.8 ng/mL	(Prevalence N/A)	Positive correlation between vitamin D and Hb (rs= 0.49, p< 0.001). Low vitamin D levels are associated with reduced Hb and ferritin levels. Further research is needed to determine whether there is a direct correlation between vitamin D insufficiency and iron deficiency anaemia.
Braithwaite et al. 2019 United Kingdom	Pregnant Women, n=195 18 years and above	All: 44.1±16.0nmol/L Placebo: 42.5±15.8nmol/L Vitamin D3 group: 45.7±16.2nmol/L	10% iron deficient (ferritin < 15 µg/L)	At late pregnancy, 25(OH) D concentration was positively associated with hepcidin in placebo group (p= 0.006) but not in vitamin D3 group (p= 0.63). There were also positively associated between 25(OH)D concentration and ferritin in placebo group (p<0.001) but not in vitamin D3 group (p= 0.19).

Michalski et al. 2017 Vietnam	Adults (women) n=4961, 18-40 years (mean ages: 26 years	57.4±10.7 nmol/L	21%	Vitamin D intakes significant correlated with Hb concentration (r= 0.03) but no longer correlate after adjustment of sociodemographic (p= 0.56). Women with serum 25 (OH) D 50nmol/L (p= 0.03). Low food sources of dietary vitamin D. Limited resources on measuring serum 25 (OH) D concentration.
Madar et al. 2016 Norway	Adults n=251 18-50 years	29±17.6 nmol/L	Anaemia: 14% (females) 5% (males)	No significant correlation between serum 25(OH) D and SR (r= 0.06), Hb (r= 0.04), SI (r=-0.05), TS (r= -0.004). There was no difference in serum folic acid or serum vitamin B12 levels after using vitamin 10 and 25 µg of D3 supplements.
Yuan et al. 2017 China	Pregnant Women n=4718	39.9 (gestational anaemia) 44.3 (control)	27%	Maternal serum 25(OH)D concentration significant lower in women with anaemia (P<0.001). Women with 25(OH)D concentration <0.25nmol/L has s increase risk of gestational anaemia compared with women of concentration >50.0nmol/L. The lower the serum 25(OH)D concentration, the greater risk of anaemia (p= 0.012).
Kim et al. 2016 Seoul, Korea	Healthy patients with ESRD n= 410 18 years and above	11.1±6.4 ng/mL	Hb: 69.9%	Hb concentration was significantly correlated with serum 25(OH)D (r=0.292, p<0.001). Anaemia was found to be significantly associated with 25(OH)D3 deficiency in patients with end-stage renal disease. It is necessary to conduct

				randomised controlled studies to evaluate whether vitamin D administration can ameliorate anaemia in these individuals.
Hennigar et al. 2016 United States	n= 152 18 - 42 years	Placebo: 5.25±3.45 µg/d Vitamin D3 group: 27.8±5.13 µg/d	Hb Placebo: 124±11.0 g/l Hb Ca + Vit D: 127±9.0 g/l	The treatment group (snack bar with vitamin D) had higher serum iCA ($P< 0.05$) and lower PTH ($P< 0.05$) after BCT compared to placebo group (snack bar without vitamin D). Ferum status were no different in both groups indicate no association with vitamin D intake.
Grübler et al. 2016 Australia	n= 200 18 years and above with serum 25OHD concentration below 75 nmol/L in hypertensive patients	25(OH)D Placebo Baseline: 50.8±14.2nmol/L Placebo Intervention: 59.0±22.1nmol/L Vit D Gp Baseline: 54.9±13.6 nmol/L Vit D Gp Intervention: 90.3±18.3nmol/L	Hb Baseline: 14.4±1.3 g/dl Hb Intervention: 14.3±1.3 g/dl Hb Placebo: same as baseline and after 8 weeks	Vitamin D had no significant effect on Hb levels in both group (treatment & placebo) ($p >0.999$). In non-anaemic hypertensive individuals with 25(OH)D levels less than 75 nmol/L, a daily vitamin D supplement of 2800 IU for 8 weeks was not result in an increase in Hb levels.

Thomas et al. 2015 New York	n= 158 15 - 18 years	25(OH)D Midgestational: 56.0±25.6 nmol/L Delivery: 53.4±29.1 nmol/L	3.2% in the first trimester 5.4% in the second tri-mester 20.6% in the third trimester 15.8% at delivery	There were positively significant associated between dietary intakes of iron and vitamin D (R2= 0.272, P >0.0001). Maternal 25 (OH) D positively significant associated with maternal Hb concentration both at mid gestation (R2= 0065, p= 0.005) and at delivery (R2= 0.084, p= 0001) but were negatively associated with other biomarkers

CHAPTER 3

METHODOLOGY

3.1 Study Design

A cross-sectional study was carried out to determine the associations between vitamin D-related factors (dietary, supplement usage and SEL) and Hb concentration among female students in UPM. Data including sociodemographic characteristics, dietary and supplement of vitamin D, SEL, body weight status and Hb concentration were collected during the data collection.

3.1.1 Sampling Design

Multistage sampling design was used to select the participants in this study. In total of 15 faculties in UPM Serdang were categorised into 3 fields of study which were sciences, art and social sciences technical. One faculty from each field of study was randomly selected using random sampling method. From each faculty, convenience sampling method was used to select the participants. However, due to Covid-19 pandemic, the multistage sampling method was changed to convenience sampling method due to data collection cannot be done in the respective faculties, thus, the location of the data collection has been changed to residential colleges. Out of 9 colleges in UPM Serdang, 3 colleges were selected by convenience sampling which were Kolej

Pendeta Za'ba (KPZ), Kolej Sepuluh (K10) and Kolej Tujuh Belas (K17) regardless the courses of their study. All female students who fulfil the criteria and agreed to participate were invited to partake in this study. **Figure 3.1** describes the sampling design of the study before pandemic COVID-19 while **Figure 3.2** describes the convenient sampling method that was used to recruit participants in this study.

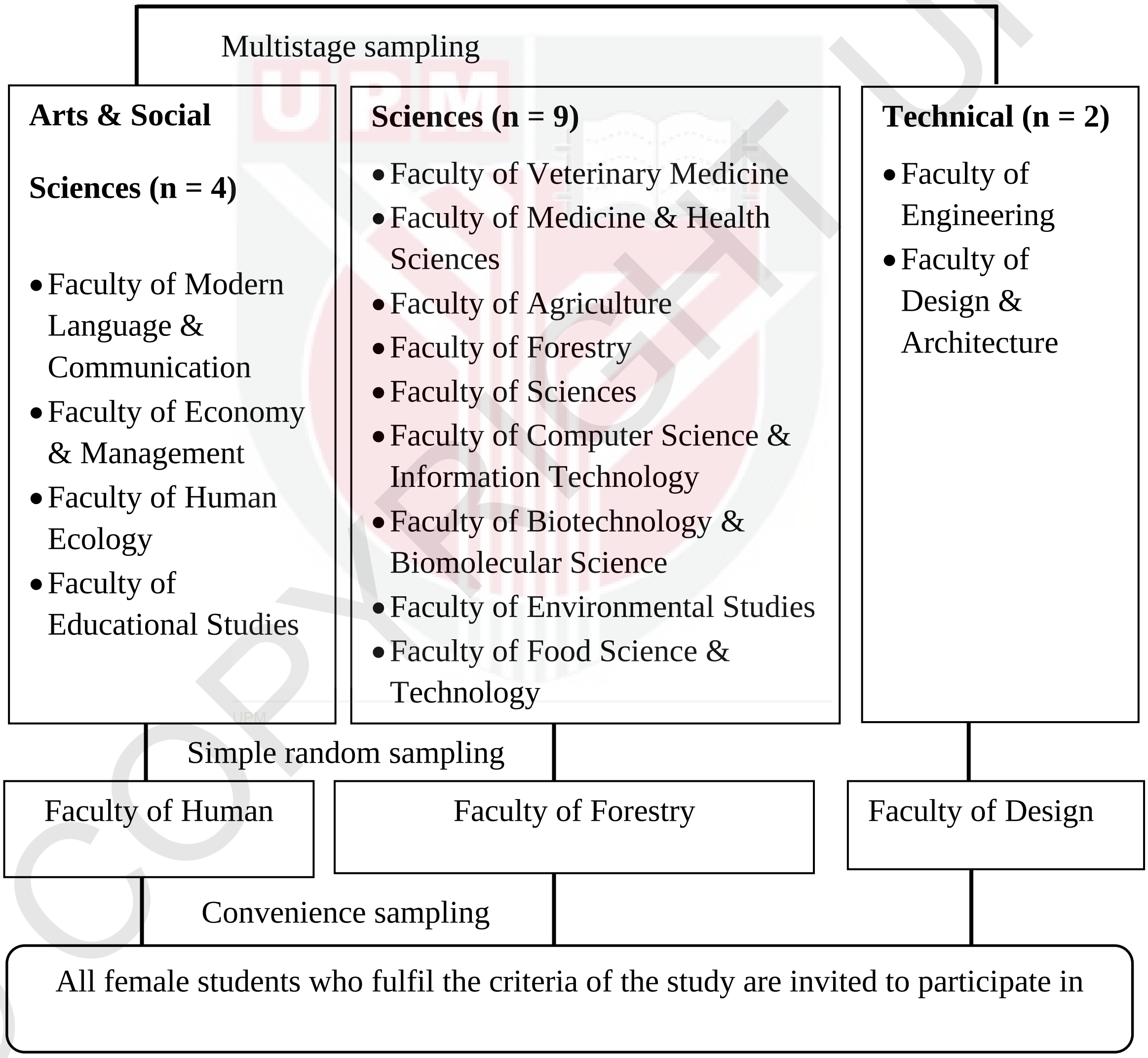


Figure 3.1 Multistage sampling design

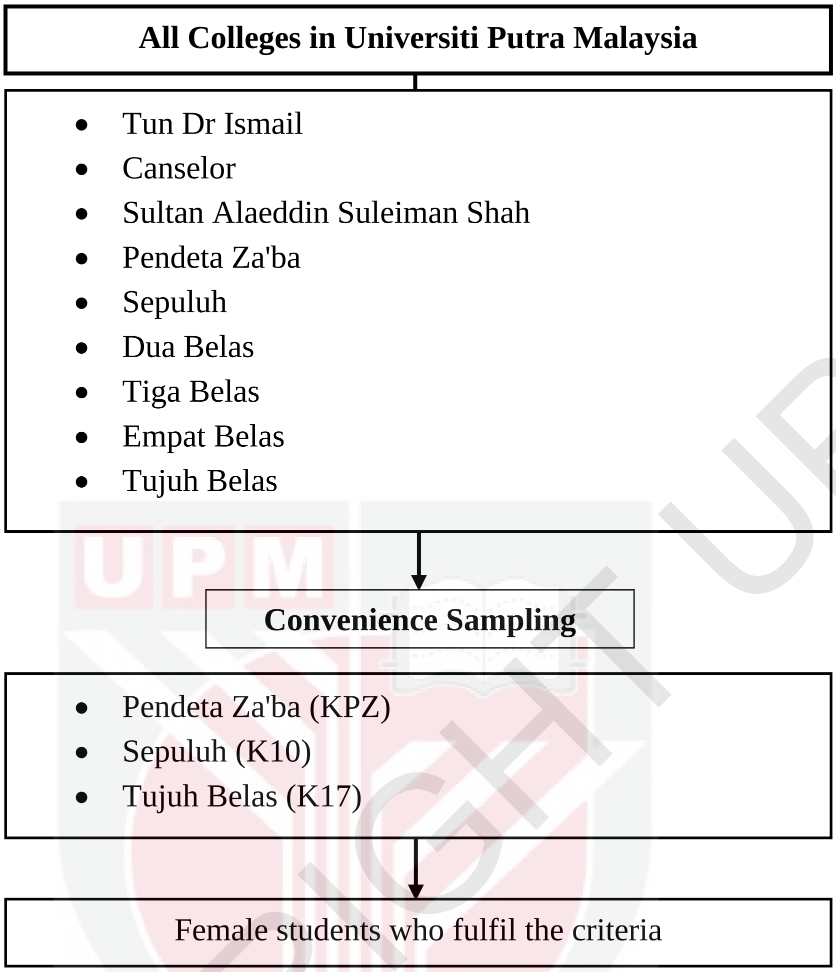


Figure 3.2 Convenience sampling design

3.1.2 Study Protocol

Figure 3.3 summarises the data collection procedures for this study. Data collection was carried out from March to April 2021. Prior to data collection, approval for the study protocol was obtained from the Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia (JKEUPM) and principle of KPZ, K10 and K17 college as shown in **Appendix A, C, D and E**. Due to the COVID-19 SOP, participants were required to observe the SOP including wearing a mask, practising social distancing and were provided with hand sanitisers before any assessment was carried out. Participants who have fever, cough or any other related covid-19 symptoms, in contact with individuals with covid-19 or waiting for swab test/results were not allowed to participate in the study. All information related to the study including the potential risks during the data collection were explained to the participants in the participant's information sheet and consent form. The consent form was used to obtain agreement from the participants before partaking in the study. The interview session was conducted approximately 30 minutes. Participants were assessed on sociodemographic, anthropometric measurement, dietary vitamin D intake, supplement vitamin D usage and SEL using vitamin D FFLQ questionnaire and sun exposure questionnaire adapted from Nurbazlin et al.

3.1.3 Study Approval

Ethical approval was obtained from the JKEUPM (Reference No: JKEUPM-2021-032) to the data collection, which followed by obtaining permissions from the principle of KPZ, K10 and K17 college of UPM.

3.2 Study Location

This study was carried out in the Kolej Pendeta Zaba' (KPZ), Kolej Sepuluh (K10) and Kolej Tujuh Belas (K17), Universiti Putra Malaysia (UPM), Serdang. The initial plan was to collect data in Faculty of Human Ecology, Faculty of Forestry and Faculty of Design and Architecture, UPM. However due to MCO, universities were not allowed to open thus the locations were changed to residential colleges which were KPZ, K10 and K17. Data collection was carried out in the Bilik Seminar (KPZ), Foyer Dewan (K10) and Dataran Kolej 17 (K17), UPM.

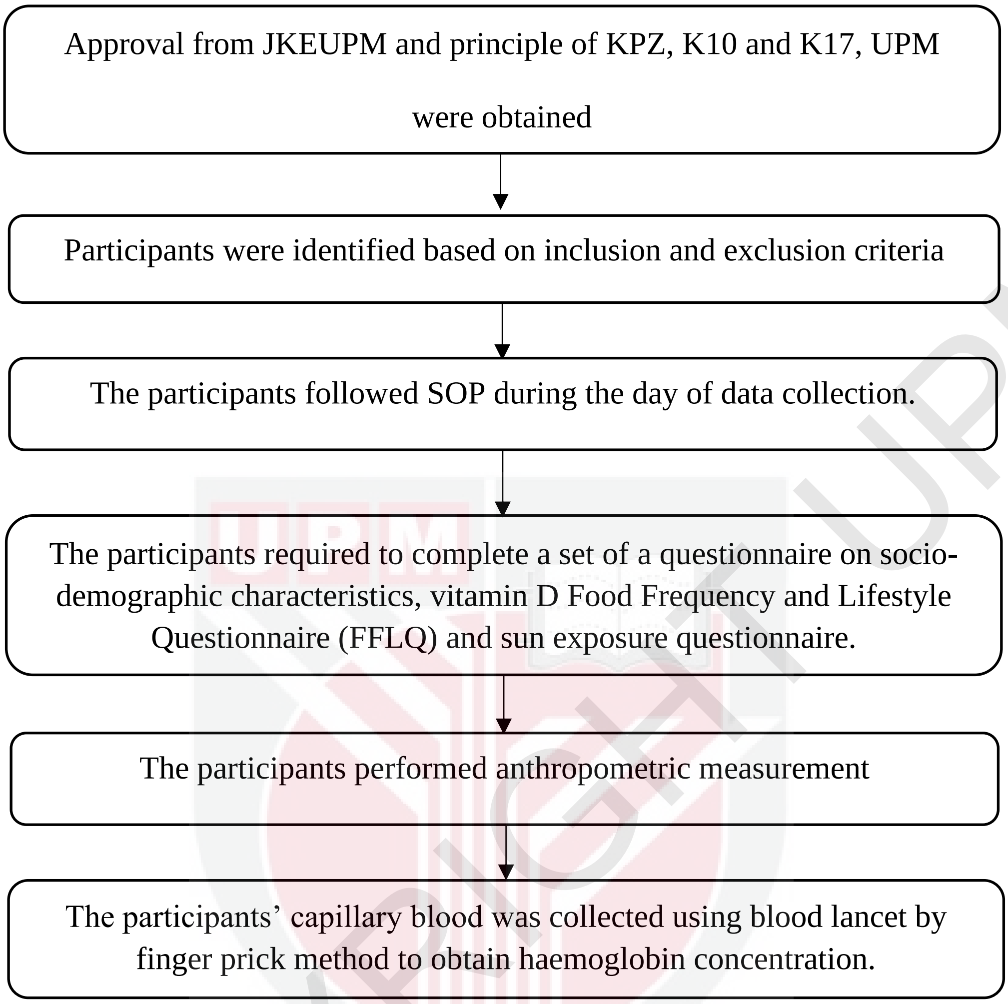


Figure 3.3 Study protocol

3.3 Participants

The participants of this study were recruited among female students in the KPZ, K10 and K17, Universiti Putra Malaysia. Participants were selected based on the inclusion and exclusion criteria as shown in Table 3.1. Female students were selected because anaemia has been shown to be more prevalent in childbearing age women, and that the prevalence was much higher in women compared to men, demonstrated in various studies carried out worldwide, including the local studies.

Table 3.1: Inclusion and exclusion criteria for selection of participants

Inclusion criteria	Exclusion criteria
• Malaysian • Female pre and undergraduate's student • Aged 18 -49 years old	• Pregnant and lactating women • Presence of chronic disease including celiac disease, gastroesophageal reflux disease (GERD), diabetes mellitus, and metabolic disorder • Donated blood for the past 6 months • Having heavy bleeding blood loss due to menstrual cycle

3.4 Sample Size Determination

The sample size required in this research studied was 146 participants. A formula by Hulley et al. (2013) was used to determine the number of participants involved.

Formula:

$$N = \left[\frac{(Z_{\alpha}+Z_{\beta})}{c}\right]^2 + 3$$

$$c = 0.5 * ln\left[\frac{(1+r)}{(1-r)}\right]$$

Where:

The standard normal deviate for Zα = 1.96

The standard normal deviate for Zβ = 0.84

The expected correlation coefficient = r

Table 3.2: Sample size calculation

Variables	Correlation, r	Sample size, n
Vitamin D and Hb (Basutkar et al., 2018)	r = 0. 496	C = 0.5 * ln [(1 + 0.496)/ (1 – 0.496)] = 0.544 N = $[(1.96 + 0.84) / 0.544]^2 + 3$ = **29**
25(OH)D3 and Hb levels (Kim et al., 2016)	r = 0.292	C = 0.5 * ln [(1+0.292) / (1-0.292)] = 0.301 N = $[(1.96 + 0.84) / (0.301)]^2 + 3$ = **90**
Vitamin D associated with iron (EL-Adawy et al., 2019)	r = 0.360	C = 0.5 * ln [(1+0.360) / (1-0.360)] = 0.377 N = $[(1.96 + 0.84) / (0.377)]^2 + 3$ = **58**

The adjustment was calculated as below using the data from Kim et al. (2016) study as shown in **Table 3.2**.

Adjustment in computing the sample size:

i) Sample Size Effect = DEFF= 1.3

n = 90 x 1.3 = **117**

ii) Response rate (80%):

$$\frac{\text{\# of respondents you need}}{\text{expected \% response rate}} \times 100$$

n = $\frac{117}{80} \times 100 = 146.25 \approx$ **146 participants**

A total of 146 participants were required for the study based on the findings from Kim et al. (2016) study with 20% drop out rate consideration.

Sample size estimated using anaemia data from a studied by Kim et al. (2016) carried out in end-stage renal disease (ESRD) patients ages between 18-70 years in Seoul, Korea. The study aimed to investigate the 25-hydroxyvitamin D3 (25(OH)D3) deficiency was associated with anaemia in ESRD patients. It was reported in the study that mean ± SD of Hb concentration was 9.9 ± 1.9. Serum 25(OH)D3 was positively correlated with Hb levels ($r=0.292$; $p<0.001$). With a sample size effect of 1.3, the total sample size required in this present study was 117. Allowing for a 20% drop out rate, the total sample size required to demonstrate a significant correlation between vitamin D related factors with Hb concentration was estimated to be 146.

3.5 Self-Administered Questionnaire

Participants were required to complete a set of questionnaires to obtain information related to sociodemographic characteristics which include age, date of birth, ethnicity, course, year of study and monthly allowance.

3.6 Anthropometric Measurements

Body weight (kg) and height (cm) were measured to determine body weight status of participants (National Health and Nutrition Survey, 1988). Body weight was measured using the SECA 803 flat weighing scale (SECA, Hamburg, Germany) to the nearest 0.1 kg (National Health and Nutrition Survey, 1988). The participants were required to remove the shoes, socks, or any heavy items from their bodies (National Health and

Nutrition Survey, 1988). The weighing scale was placed on a flat surface and zero balance was assured before taking the measurement (National Health and Nutrition Survey, 1988). A SECA 213 stadiometer (SECA, Hamburg, Germany) was used to measure the participants' height to the nearest 0.1 cm (Miranda et al., 2014). The participants were required to stand upright and barefooted on the stadiometer and the head was positioned in the Frankfort horizontal plane (Madden, 2018). After the data on body weight and height were acquired, BMI was used to classify the body weight status by using the following formula and classification in **Table 3.3.**

$$Body\ Mass\ Index\ (BMI) = \frac{body\ weight\ (kg)}{height\ (m^2)}$$

Table 3.3: Classification of BMI (WHO, 2000)

Classification	**BMI (kg/m²)**
Underweight	< 18.5
Normal	18.5 - 24.9
Overweight	25.0 – 29.9
Obesity Class I	30.0- 34.9
Obesity Class II	35.0 -39.9
Obesity Class III	>40.0

3.7 Determination of Vitamin D-Related Factors

3.7.1 Assessment of Dietary Vitamin D

Dietary of vitamin D was assessed using Vitamin D Food Frequency and Lifestyle Questionnaire (FFLQ) adapted from the Vitamin D Specific Questionnaire (Larson-

Meyer et al., 2019). The use of FFLQ questionnaire was focus on the components of dietary vitamin D which may potentially influence participants' vitamin D status. The questionnaire evaluates the consumption frequency of vitamin D-containing foods and supplements.

The Malaysian Food Composition Table (Tee et al., 1997) and Nutritionist Pro Software were used to analyse the dietary intake. The dietary vitamin D intake of participants would be estimated using the following formula where we assume 30 days per month (conversion factor: 30 days).

Vitamin D intake (µg) = frequency midpoint × average content of each vitamin D food

The amount of dietary vitamin D intake of participants is compared with Recommended Nutrient Intake (RNI) for vitamin D for adults aged 19 to 65 years which is 15 µg/day (National Coordinating Committee on Food and Nutrition, 2017). Total dietary vitamin D intake would be analysed using Nutritionist Pro Version 25 (First Data Bank Inc., 2011).

3.7.2 Assessment of Vitamin D Supplementation

The similar FFLQ questionnaire was used to assess the intake of supplement (Larson-Meyer et al., 2019). There were 4 questions to address multivitamin, vitamin D and calcium vitamin use and includes the option of specifying specific brand(s) and supplement doses if known. The amount of vitamin D from supplements were calculated using the actual dose or specified brand they reported. If they were not mentioning the brand, 600 IU as a standard for a multivitamin and 1000 IU as a standard for supplemental vitamin D (Larson-Meyer et al., 2019). Vitamin D that expressed in International Units (IU) can be converted into µg; 1 IU of vitamin D = 0.025 1µg. The supplement vitamin D intake of participants was estimated using the following formula where we assume 30 days per month (conversion factor: 30 days).

Vitamin D supplement intake (µg) =

frequency midpoint × average content of vitamin D in supplement

3.7.3 Sun Exposure Level Assessment

SEL was assessed using a questionnaire adapted from Nurbazlin et al. (2013). Participants were asked about their outdoor activities over the previous week in terms of duration in minutes and frequency per week, sunscreen and umbrellas frequency usage. Duration of outdoor activities is taken from 7 am to 7 pm based on the sun rise and sun set time in Malaysia. The usage of sunscreen and umbrella were measure by frequency of always, sometimes or never wear during outdoor activity. The sun index, which is an index combining a measure of time outdoors during daylight and BSA usually exposed during that time. The adapted "Rule of Nine" was used to estimate the fraction of body surface area (BSA) exposed to sunlight by the participant's attire during outdoor activity. This method divides the body's surface area into percentages of face (9%), both hand (9%), full arm (18%), half arm (9%), full legs (36%), half legs (18%) and other surface (1%). The sun exposure index (SEI) was calculated as follows:

SEI = (hours of sun exposure per week) x (fraction of BSA exposed to sunlight)

Hours of sun exposure per week was calculated by adding up total hours they spend outdoors and multiply with frequency they go out. SEL/week, BSA fraction and SEI was used as a measure of SEL.

Table 3.4: BSA exposed to sunlight

		Category 1			**Category 2**		**Category 3**	
	Adapted "rule of nines"[a]	**No shirt**	**Long-sleeved shirt**	**Short-sleeved shirt**	**Short pants**	**Long pants**	**No hat**	**Hat**
Both arms	0.18	0.18	0.04	0.14				
Both legs	0.36				0.24	0.00		
Anterior trunk	0.18	0.09	0.00	0.00	0.00	0.00		
Posterior trunk	0.18	0.09	0.00	0.00	0.00	0.00		
Head	0.09						0.07	0.03
Perineum	0.01				0.00	0.00		
Column totals	1.00	0.36	0.04	0.14	0.24	0.00	0.07	0.03

"The rule of nines estimates sectors of adult BSA as percentages that are multiples of 9.

The UV Index Cut Off from WHO (2002) was used to categorize the SEI level of participants according to UV Index range as shown as Table 3.5.

Table 3.5: UV index cut off point (WHO, 2002)

Exposure category	**UVI range**
Low	<2
Moderate	3-5
High	6-7
Very High	8-10
Extreme	11+

3.8 Haemoglobin Concentration Assessment

Hb concentration were collected using HemoCue© Hb 201+ Analyzer (HemoCue AB, Ängelholm, Sweden). Blood samples were obtained by a finger-prick method using blood lancet by the researcher. The researcher has been trained to do finger prick before collecting the data. The process began with cleaning the middle finger with alcohol swab before the prick was obtained. The first drop of blood was cleaned off and tip of the finger was squeezed to collect the second drop of blood. The collected blood sample was placed in the micro cuvette and in the cuvette holder to measure the Hb concentration. The Hb concentration was classified using the cut off by WHO (2000) as shown in **Table 3.6**.

Table 3.6: Hb levels to diagnose anaemia (g/dl)

Population	**Non-anaemia**	Anaemia		
		Mild	**Moderate**	**Severe**
Non-pregnant women (15 years of age and above)	12.0 or higher	11.0-11.9	8.0-10.9	lower than 8.0

WHO (2000)

3.9 Pre-Testing

Pre-testing of the questionnaire was conducted before the actual data collected was carried out. A total of 30 female students from UPM with the similar criteria as the study sample and fulfil the inclusion and exclusion criteria of this study were recruited as participants for the pre-testing. Participants who were involved in the pre-testing were not be included in the actual data collection. Pre-testing was carried out to ensure that questions being asked were suitable to get the desired information and used to estimate the time required by participants to answer the questionnaire. Improvement of the questionnaire was carried out based on the feedback received from the participants. The reassessment on the feasibility of the questionnaires was carried out after the pre-testing process.

3.10 Statistical Analysis

IBM SPS 25.0 for Windows Version (IBM Corp., Armonk, NY) was used for the statistical analysis. The normality of all variables was determined by using skewness test. The data for continuous variables were presented as mean and standard deviation (SD) whilst data originates from the categorical variable were presented as frequency and percentages (%). Correlation test (Pearson correlation and Spearman correlation) were used to determine the associations between vitamin D-related factors (dietary, supplement usage and SEL) and Hb concentration as appropriate. The level of significance was set at $p<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Socio-Demographic Characteristics

Table 4.1 shows the socio-demographic characteristics of the participants. The participants' mean ± SD age of participants was 22.4 ± 1.34 years. The majority of the participants were 23 years (36.1%). Approximately 78.1% of participants were Malays, 11.0% were Chinese, 6.5% were Indian, whilst the remaining 4.5% were other races. The majority of the participants were 4th year students (38.1%), followed by 3rd year (25.2%), 2nd year (17.4%), 1st year (14.2%), 5th year (3.2%) and the least were Asasi students (1.9%). Majority of the participants were from K17 college (38.1%), followed by KPZ college (36.9%) and K10 college (25.8%). The mean ± SD monthly allowance of participants was RM 409.19 ± 246.6. Approximately all participants (98.7%) did not involve in exercise for more than 4 hours per week.

Table 4.1: Distributions of participants by socio-demographic characteristics (n= 155)

	Mean ± SD	n	(%)
Age	22.40 ± 1.34		
Ethnicity			
Malay		121	78.1
Chinese		17	11.0
Indian		10	6.5
Others		7	4.5
Year of Study			
1st Year		22	14.2
2nd Year		27	17.4
3rd Year		39	25.2
4th Year		59	38.1
5th Year		5	3.2
Asasi		3	1.9
Student's Residential College			
KPZ		56	36.1
K17		59	38.1
K11		40	25.8
Monthly Allowance (RM)	409.19 ± 246.60		
Engaged in Excessive Exercise (>4h/Week)			
Yes		2	1.3
No		153	98.7

4.2 Anthropometric Measurements

Table 4.2 shows the distributions of participants by body weight status. The mean ± SD of participants' BMI was 22.6 ± 4.7 kg/m^2, with majority of the participants had a normal BMI (56.8%), followed by underweight (18.7%) and overweight (14.8%). The remaining participants were considered obese (9.7%). None of the participants were classified in obesity class II and III.

Table 4.2: Distributions of participants by BMI (n=155)

	Mean ± SD	n	(%)
BMI (kg/m^2)	22.6 ± 4.7		
Underweight (<18.5)		29	18.7
Normal (18.5-24.9)		88	56.8
Overweight (25-29.9)		23	14.8
Obesity Class I (30-34.9)		15	9.7

The finding was almost similar to another study carried out by Gan, Mohd Nasir, Zalilah, & Hazizi (2011), which examined differences in eating behaviours, dietary intake, weight status, and body composition among 584 students aged 20.6 ± 1.4 years from four Malaysian universities in the Klang Valley. It was found that 22.4% of females were underweight whilst 12.3% were overweight and obese. Another local study carried out among students in Universiti Sultan Zainal Abidin (UniSZA) carried out by Fatehah et al. (2018) reported that there were 23.0% of female students were overweight and 11.0% of the students were obese. The prevalence of overweight and obesity classes I-III is 18% and 9%, respectively, among female respondents, compared to 4% and 7%, respectively, among male respondents. (Fatehah et al., 2018). On the other hand, a study carried out by Huda and Ahmad (2010) that was aimed to measure the nutritional status through BMI profile among 624 Universiti Sains Malaysia (USM) students, found that 60.6% of female students were normal, underweight (32.8%) overweight (6.1%) and obese (0.6%).

Another local study carried out among medical students of AIMST University to determine the prevalence of overweight and obesity found 14.8% of 290 students were found overweight (BMI 23-24.9 kg/m^2) with pre-obese (BMI 25-29 kg/m^2) made up to

15.9% and 5.2% were determined to be obese (Gopalakrishnan et al., 2012). Nearly, 63.8% of the undergraduates in the study were Indian, 32.4% were Chinese and 3.8% were Malay (Gopalakrishnan et al., 2012).

Another local study carried out among students in five public universities found that there were 23.0% of bachelor students were overweight and 17.6% of the students were obese (Wan Mohamed Redzi et al., 2019). A study carried out by Koo et al. (2019) among Malaysian university students in private university in Klang Valley reported that there were 29.3% of female students were overweight whilst 19.2% were obese. The findings of this study may be contradicted with other local study findings due to the BMI classification guideline used where the present study have used WHO BMI classification whilst other study used Asian BMI classification (Pourzand et al., 1999).

4.3 Haemoglobin Concentration

Table 4.3 shows the distributions of participants by Hb concentration. The mean ± SD of Hb concentration was 12.10 ± 1.38 g/dl. ~~The~~ majority of the participants were not anaemic (61.3%) with the prevalence of anaemia was 38.7%. In terms of severity, participants with mild anaemia were 23.9% and moderate anaemia were 14.8%. None of the participant was severely anaemic.

Table 4.3: Distribution of participants by Hb concentration (n=155)

	Mean ± SD	**n**	**(%)**
Type of Anaemia (g/dl)	12.10 ± 1.38		
Moderate Anaemia (8.0-10.9)		23	(14.8)
Mild Anaemia (11.0-11.9)		37	(23.9)
Non-Anaemia (>12.0)		95	(61.3)

In the present study, the prevalence of anaemia observed was 38.7% which was slightly higher with the result reported by WHO with 30.1% of Malaysian adult classified as anaemic (McLean et al., 2009). National Health and Morbidity Survey (NHMS) (2015) also reported almost similar prevalence of anaemia with WHO data which was 35.5% (Institute for Public Health, 2015). A local study carried out among Malaysian adults reported that prevalence of anaemia among non-pregnant women aged 15 to 49 years was 34.75% (Awaluddin et al., 2017). The result was almost similar with a study carried out in Saudi Arabia among female undergraduate university students which reported an iron deficiency anaemia (IDA) prevalence of 35.3% (Jamea et al., 2019). IDA was the leading cause of anaemia in the developed country according to a review on the burden of disease in 187 countries between 1990 and 2010 (Kassebaum et al., 2014).

In contrast to another study carried out by Azma et al. (2012) that was aimed to determine IDA prevalence and thalassemia among 400 medical students in Universiti Kebangsaan Malaysia, Kuala Lumpur, it was showed that 9.9% of female students was anaemic with Hb level of 9.2 – 11.9 g/dL whilst 5% of female students was classified as IDA. Another study carried out among university students in Bangladesh's Noakhali Region discovered that 55.3% were anaemic, with 63.3% of female students being anaemic. Students aged 20-22 years had a higher rate of anaemia (43.4%) than other age groups (Shill et al., 2014).

The high prevalence of anaemia in the current study could be attributed to the life style of female students as well as to their dietary patterns (Al Hassand, 2015). Studies have demonstrated that, anaemia was more prevalent among the students who skipped breakfast (Abalkhail & Shawky, 2002). Breakfast is generally known to be ignored and skipped more frequently by teenagers and young adults than by any other age group (Al Hassand, 2015). In a study carried out by Moy et al. (2014) found high prevalent of skipping breakfast by 29.2% among university students in Kuala Lumpur. Although the current study did not analyse the participants' eating habits, some of the data collection was done in the morning, thus some of the participants may have skipped breakfast before data collection.

Anaemia affects all population groups and appears to maintain contributing to the global burden of disease if no intervention or action is taken quickly (Awaluddin et al., 2017). WHO (2008) reported that the prevalence of anaemia in Malaysia were considered as moderate public health problem (McLean et al., 2009). The National Plan of Action for Nutrition of Malaysia (NPANM) III, 2016-2025 has included intervention plan to achieve a 50% reduction in the prevalence of anaemia in women of reproductive age by 2025.

4.4 Dietary of Vitamin D Intake

4.4.1 Mean Dietary Vitamin D Intake among Participants

Table 4.4 shows the dietary vitamin D intake of participants assessed using FFLQ. The mean ± SD vitamin D was analysed as one of the dietary confounders that could potentially affecting Hb level. The mean of dietary vitamin D intake was low 8.10 µg/day as a result of participants' potential underestimation of serving size. Majority of the participants had vitamin D intake below recommended dietary intake (<15 µg).

Table 4.4: Distribution of participants by dietary vitamin D intake (n=155)

	Mean ± SD	**n**	**(%)**
Dietary Vitamin D	8.10 ± 5.08		
<15 µg/day		132	85.2
>15 µg/day		23	14.8

The findings of a prior study among pregnant women, and adolescents were consistent with the findings of the current study, with the majority of respondents unable to meet Malaysia's RNI for dietary vitamin D (Zaleha et al., 2015; Suriawati et al., 2016). The finding was almost similar with a cross sectional study carried out by Jamil et al. (2019) that was aimed to assess knowledge, attitude and practice (KAP) related to vitamin D and its association with vitamin D status among 147 Malay women office workers aged between 20–55 years in Kuala Lumpur (Jamil et al., 2019). It was found that 94% of participants had low median dietary vitamin D intake (5.2 µg/day) than the RNI (Jamil et al., 2019). Another study was carried out aimed to determine total daily dietary intakes from food only and food plus supplements among women in childbearing age (WCBA) and women of menopausal age (WMENO). It was found mean ± SD vitamin D intake from food alone was 3.8 ± 0.1 µg in WCBA aged 15–30 which was lower than recommendation (Devarshi et al., 2021). The current study reported mean of dietary vitamin D intake lower compared to data from 2011–2016 NHANES among WCBA (Devarshi et al., 2021). Another study compared the total vitamin D intake from diet of Asians residing in Kuala Lumpur and Aberdeen and found median vitamin D intake was only 1.37 µg/day (Jamil et al., 2018).

Previously, 25(OH)D has been reported as the primary biomarker for detecting vitamin D status in various research. Overall, the mean 25(OH)D concentration was 69 nmol/L in a study, with females (64 nmol/L) having a lower concentration than males (74 nmol/L) ($p<0.001$) (Nimitphong & Holick, 2013). According to Moy and Bulgiba's (2011) study among 380 Malay adults in Kuala Lumpur to determine the prevalence of vitamin D deficiency and the association of 25(OH)D with metabolic risk factors found that 87% of females had 25(OH)D levels below 50nmol/L ($p< 0.001$) (Moy & Bulgiba, 2011). In a study conducted to examine vitamin D status among 372 Cameroonians adults from the South West Region, as well as the risk factors associated with it found nearly 25.8% of participants had vitamin D deficiency (Tangoh et al., 2018). In a further study, the status of vitamin D among Chinese and Malay men in Malaysia and its associated factors were found to be more widespread in Malaysia compared to Chinese ($p< 0.05$) and serum vitamin D levels significantly lower (<50 nmol/L) were in people aged between 20 and 29 years compared to those aged over 40 years old ($p < 0.05$) (Chin et al., 2014). However, in the present study, we did not assess the association between sociodemographic with vitamin D factor.

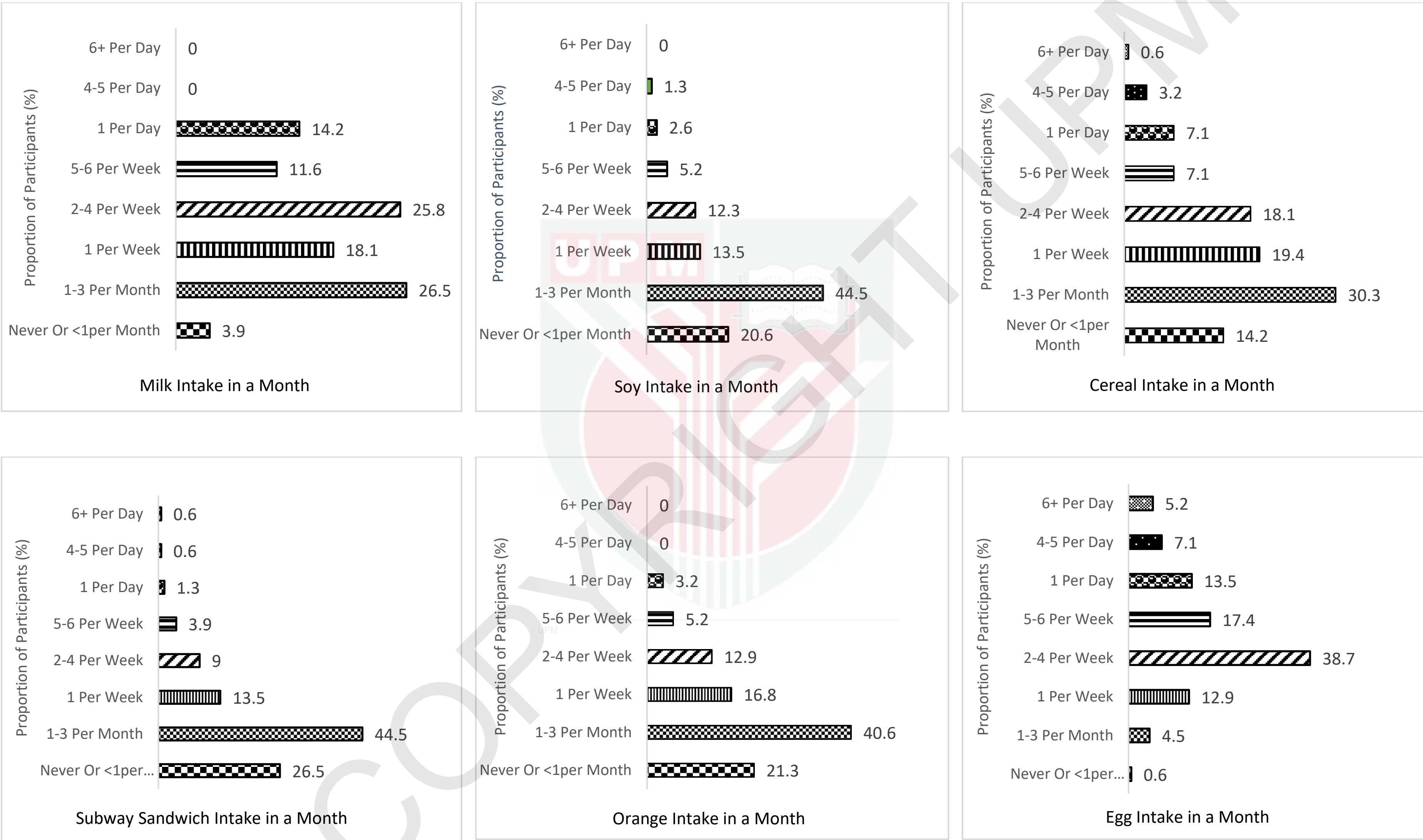
Table 4.5 shows mean ± SD distributions of vitamin D intake by food item assessed by FFLQ questionnaire. The result showed that the mean ± SD vitamin D intake of sardine was the highest (1.22 ± 1.56 µg/day) followed by cod liver oil (1.16 ± 2.96 µg/day) and mackerel (0.88 ± 1.30 µg/day) approximately to 15.1%, 14.4% and 10.8% respectively. Subway sandwich was the least of vitamin D intake in a month (0.02 ± 0.06 µg/day). This was almost similar with another study aimed to determine the effect of dietary of vitamin D levels in Asians residing in KL and Aberdeen and found fresh oily fish provided the most vitamin D in the diet, accounting for 59% in Kuala Lumpur, followed by 22% of eggs and 14% of sardines and tuna respectively (Jamil et al., 2018). The mean of dietary vitamin D intake was low compared to RNI could be due to less frequent intake of oily fish among Malaysian population (Jamil et al., 2018). Apart from that, vitamin D fortification is optional in Malaysia and is limited to a small number of dairy products and breakfast cereals (Jamil et al., 2019) Moreover, Malaysians consume relatively less dairy products in daily life which will result in low mean of vitamin D intake (Nguyen Bao et al., 2018). Besides, in Malaysia, vitamin D fortification is optional and limited to select dairy products and breakfast cereals (Jamil et al., 2019). Yet, dairy products consumption among Malaysians was generally low (Nguyen Bao et al., 2018).

Table 4.5: Distribution of vitamin D intake by food item in a month (n=155)

Foods (µg/day)	**Vitamin D Intake in Food Item**	
	Mean ± SD	**(%)**
Milk	0.49 ± 0.43	6.0%
Soy	0.57 ± 1.36	7.1%
Cereal	0.46 ± 0.97	5.7%
Subway Sandwich	0.02 ± 0.06	0.3%
Margarine	0.24 ± 0.59	3.0%
Orange	0.46 ± 0.61	5.6%
Chicken Liver	0.09 ± 0.46	1.2%
Egg	0.71 ± 1.02	8.7%
Cod liver Oil	1.16 ± 2.96	14.4%
Salmon	0.85 ± 1.24	10.5%
Mackerel	0.88 ± 1.30	10.8%
Sardine	1.22 ± 1.56	15.1%
Eel	0.24 ± 0.58	3.0%
Tuna	0.70 ± 1.16	8.7%

4.4.2 Distributions Frequency of Dietary Vitamin D Intake by Food Item

Figure 4.1 shows frequency of each type of food in a month. From the figure, cod liver oil was the least consume in a month (83.2%) followed by eel (79.4%) and salmon (60.6%). The most frequent food consumed in a month (total of 1 per day, 4-5 per day and 6+ per day) were egg (25.8%), milk (14.2%) and cereal (10.9%). Another study carried out by Mikolajczyk et al. (2009) females were slightly more commonly to consume fresh fruits, salads, cooked vegetables, milk products, and cereals.


Proportion of Participants (%)
6+ Per Day 0
4-5 Per Day 0
1 Per Day 14.2
5-6 Per Week 11.6
2-4 Per Week 25.8
1 Per Week 18.1
1-3 Per Month 26.5
Never Or <1per Month 3.9
Milk Intake in a Month
Proportion of Participants (%)
6+ Per Day 0
4-5 Per Day 1.3
1 Per Day 2.6
5-6 Per Week 5.2
2-4 Per Week 12.3
1 Per Week 13.5
1-3 Per Month 44.5
Never Or <1per Month 20.6
Soy Intake in a Month
Proportion of Participants (%)
6+ Per Day 0.6
4-5 Per Day 3.2
1 Per Day 7.1
5-6 Per Week 7.1
2-4 Per Week 18.1
1 Per Week 19.4
1-3 Per Month 30.3
Never Or <1per Month 14.2
Cereal Intake in a Month
Proportion of Participants (%)
6+ Per Day 0.6
4-5 Per Day 0.6
1 Per Day 1.3
5-6 Per Week 3.9
2-4 Per Week 9
1 Per Week 13.5
1-3 Per Month 44.5
Never Or <1per... 26.5
Subway Sandwich Intake in a Month
Proportion of Participants (%)
6+ Per Day 0
4-5 Per Day 0
1 Per Day 3.2
5-6 Per Week 5.2
2-4 Per Week 12.9
1 Per Week 16.8
1-3 Per Month 40.6
Never Or <1per Month 21.3
Orange Intake in a Month
Proportion of Participants (%)
6+ Per Day 5.2
4-5 Per Day 7.1
1 Per Day 13.5
5-6 Per Week 17.4
2-4 Per Week 38.7
1 Per Week 12.9
1-3 Per Month 4.5
Never Or <1per... 0.6
Egg Intake in a Month

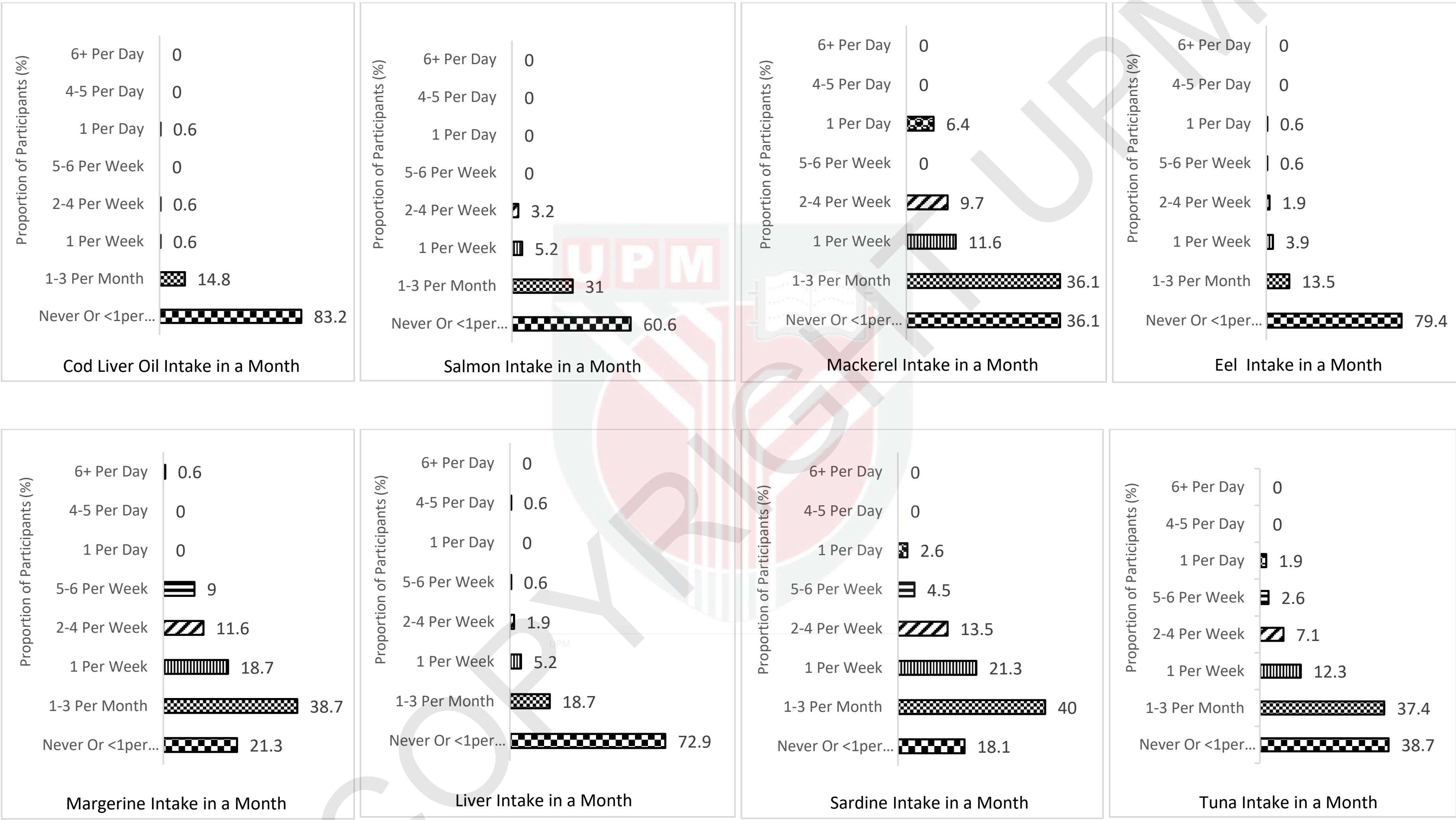


Figure 4.1 Distribution frequency of dietary vitamin D intake by food item in a month

4.5 Supplement Vitamin D Intake

Table 4.6 summarised the supplement vitamin D intake of participants. In the present study, supplement vitamin D intake was calculated as µg/day (1 IU/40). From 155 participants, 21.9% consumed vitamin D supplement from multivitamin and/or vitamin D supplementation (n=34). The results are presented as mean ± SD and shows majority of the participants did not meet the recommended RNI for vitamin D intake from supplement (87.7%). However, the present study did not assess the total amount of vitamin D intake from diet and supplement which could affect the prevalence of adequate intake of vitamin D.

Table 4.6: Mean distribution of supplement vitamin intake (n=34)

	Mean ± SD	n	(%)
Supplement Vitamin D	13.56 ± 6.38		
<15µg/day		15	44.1
>15µg/day		19	55.9

The mean ± SD intake of supplement vitamin D was 13.56 ± 6.38, with 21.9% taking vitamin D. A study carried out by Bailey et al. (2010) aimed to assess vitamin D intake from food and supplements and calcium intakes from food, water, nutritional supplements and antacids by using NHANES 2005–2006 data among U.S. citizens aged more than 1 year (Bailey et al., 2010). It was found males 14–18 year and females 19–30 year had the lowest prevalence of use of vitamin D dietary supplements (Bailey et al., 2010). In a cross sectional study carried out among university student in Pakistan it was found that 36.7% of the participants were taking supplements or multivitamin containing vitamin D with significantly higher among female students (52%) (p= .003) (Tariq et al., 2020a). In a study carried out in Malaysia aimed to examine the knowledge, attitude, and practice (KAP) of Malay female office employees regarding vitamin D and its association to vitamin D level found 90.5% were rarely or never taking vitamin D supplementation suggesting taking dietary vitamin supplements had been less popular among the research population of this study which were similar with the current study (Jamil et al., 2019).

The low mean vitamin D supplementation intake in this study could be due to participants were unaware of the vitamin D dose they were receiving (Tariq et al., 2020b). In a study carried out among university students in Pakistan, majority of the supplement vitamin D users were unaware with the dose taken with 55% who were taking vitamin D supplements reported taking less than 1000 IU (Tariq et al., 2020b). Another study carried out among Malaysian adults at Klang Valley area were found majority of the them (70%) were never had taken vitamin D supplement before (Blebil et al., 2019). According to this study, taking vitamin D supplements is associated with higher knowledge scores, indicating that when people are aware of the risks of vitamin D insufficiency, they are more likely to taking vitamin D supplement (Blebil et al., 2019).

4.6 Sun Exposure Level

Table 4.7 shows SEL characteristics of participants. The majority of the participants exposed to sunlight less than 2 hours (67.7%). Mean ± SD of body surface area (BSA) fraction exposed was 0.30 ± 0.22. The majority of participants exposed face, hands and others (feet) when going outside under the sun. The calculated SEI (SEL/week x BSA fraction) was low with mean ± SD (2.50 ± 1.46) with 78.1 % having low SEI (UV <2). For sunscreen usage, majority of the participants always used sunscreen but occasionally used umbrella or stay in shade when exposed to sunlight (54.8%).

Table 4.7: Sun exposure characteristics of participants (n=155)

	Mean ± SD	**n**	**(%)**
Duration Sun Exposure (SEL/week)	2.08 ± 1.11		
1 min – 2 hours		105	67.7
2.01-4 hours		48	31.0
4.01-6 hours		2	1.3
BSA fraction	0.30 ± 0.22		
Face, Hands, Others		102	65.8
Face, Hands, Half Arm, Others		28	18.1
Face, Hands, Full Arm, Others		5	3.2
Face, Hands, Half Arm, Half Legs, Others		10	6.5
Face, Hands, Full Arm, Half Legs, Others		8	5.2
Face, Hands, Full Arm, Full Leg, Others		2	1.3
Sun Exposure Index (SEI)	2.50 ± 1.46		
Low (UV <2)		113	72.9
Moderate (UV 3-5)		19	12.3
High (UV 6-7)		18	11.6
Very High (8-10)		5	3.2
How Often Wear Sunscreen			
Never		11	7.1
Sometimes		59	38.1
Always		85	54.8
How Often Stay in Shade or Under Umbrella			
Never		9	5.8
Sometimes		85	54.8
Always		61	39.4

Hours of sun exposure, BSA fraction and SEI were the principal predictors of SEL in the present study. The result shows that the mean ± SD of SEI was 2.50 ± 1.46 which was considered low based on the cut off by WHO (2002). The findings were similar with a study carried out by Shafinaz, and Moy (2016), which examine vitamin D levels and their association with adiposity in 858 multi-ethnic people in Malaysia and was found that discovered that the SEI among female adults was low (Shafinaz & Moy, 2016). Nearly 67.7% of our participants spent outside for 1 minutes to 2 hours per day, followed by between 2.01 to 4 hours (31.0%) and 4.01 to 6 hours (1.3%).

Majority of participants exposed face and hands when outside sun and the body surface area (BSA) fraction that exposed to sunlight was low (0.30 ± 0.22). Nurbazlin et al. (2013) reported almost similar finding where urban women in this study spent 2.92 hours/week under sun, with 21.0% of BSA exposed to the sun. As for the use of sunscreen, the majority (54.8%) of the participants always applied sunscreen, 38.1% reported sometimes sunscreen application and 7.1% never used any sunscreen when exposed to the sun. Approximately 54.8%, 39.4% and 5.8% were occasionally, never and always staying in shade or under umbrella, respectively. In another study carried out among 380 individuals in Malaysia to assess vitamin D status and its related variables, Moy (2011) reported that the sun protection score (containing the total time head covered, the use of sunscreen, long sleeve and long trousers) among females were high with mean ± SD 3.2 ± 0.9 compared to male (1.7 ± 0.7). Despite the fact that Malaysia has abundant of sunlight all year, our data demonstrated that the majority of our Malay individuals have low vitamin D levels. This has raised worries that individuals who spend the majority of their time outside in the shade and using sunscreen have a detrimental effect on vitamin D status (Moy, 2011). UVB intensity can be reduced by up to 50% in some shady environments (Turnbull et al., 2005)

In this study, we did not assessed factor associated with sun exposure avoidance. Study by Tariq, Khan & Basharat (2020) identified hot weather was the most common cause (57%) by the majority, while indoor lifestyle (31%) and cosmetic concerns (29%) were also mentioned as prominent reasons for avoiding sun exposure. Since most of our participants was from Malay ethnicity, they highly used of scarves, long sleeves and long pants as the clothing styles of Muslim which may cause low fraction in exposing body surface area (BSA). Chin, Jamil, and Nor (2018) reported similar possible explanation on low SEI among healthy adults in health facility in Malaysia.

4.7 Associations Between Vitamin D-Related Factors (Dietary, Supplement Usage and SEL) and Hb Concentration

Table 4.8 shows associations between vitamin D-related factors (dietary, supplement usage and SEL) and Hb concentration. It was observed in the present study that there were significant associations between both dietary, supplement vitamin D intake and Hb concentration. A low significant association between dietary vitamin D ($r= 0.28$, $p= 0.010$) and moderate significant association between supplement vitamin D ($r= 0.191$, $p= 0.017$) and Hb concentration were found in the present study. However, there were no significant associations found between hours sun exposure per week, BSA fraction and SEI and Hb concentration ($r= 0.098$, $p= 0.227$; $r= 0.063$, $p= 0.433$; $r=0.009$, $p= 0.912$).

Table 4.8: Associations between vitamin D-related factors and Hb concentration (n=155)

Vitamin D Factors	**Hb Concentration (g/dL)**	
	Pearson/Spearman coefficient (r)	***p* value**
Dietary Vitamin D	0.280	**0.010***
Supplement Vitamin D	0.400	**0.019***
Duration Sun Exposure (SEL/week)	0.098	0.227
BSA Fraction	0.063	0.433
Sun Exposure Index (SEI)	0.009	0.912

* Correlation is significant at the 0.05 level (2-tailed)

These findings suggest that the higher the dietary and supplemental of vitamin D intake, the higher the Hb concentration. However, the associations were weak, which could be explained by recall bias (over or underestimate) of nutritional intake and sun exposure among research participants, as these variables were obtained subjectively via questionnaires.

Similar trends of study was found in a study carried out among pre and post-menopausal women in the lowest 25(OH)D group had a significantly increased risk of anaemia after controlling for confounding variables (p= 0.009, p= 0.038), respectively (Shin & Shim, 2013). The study also reported vitamin D levels were found to be higher in postmenopausal women (≤13.36 ng/ml) when compared to pre-menopausal women (≤11.92 ng/ml). It was suggested that higher vitamin D level can be due to vitamin D supplementation as postmenopausal women consume them for bone metabolism (Shin & Shim, 2013).

Another study examined the effect of 8 weeks of iron fortified milk supplementation on iron reserves in a placebo-controlled, double-blind RCT found Hb concentration and haematocrit level of the vitamin D group was substantially higher than the placebo group ($p= 0{\cdot}037$, $p= 0{\cdot}032$) (Salma & Mushtaq, 2019). These two indicators were shown to improve in the vitamin D group whilst decreasing in the placebo group, implying that daily consumption of iron fortified cereal in combination with vitamin D supplements, rather than iron fortified cereal alone, may have an additional desirable effect on iron level (Salma & Mushtaq, 2019).

In another study demonstrated a substantial correlation between albumin and vitamin D insufficiency and an increased anaemia incidence, significant lower Hb, and increased usage of erythrocyte-stimulating drugs in anaemia patients ($r=0.21$; $p<0.01$) (Sim et al., 2010). The prevalence of anaemia in those with D25 deficiency was higher 48% than 36% in the normal D25 group. The study indicate that anaemia may have lead patients to D25 insufficiency, since anaemic patients may have been less probable to walk outside and acquire appropriate sun exposure due to tiredness (Sim et al., 2010).

No associations found between SEL factors and Hb concentration could be due to low exposure of the sun. Since the data collection held during COVID-19 pandemic, less participants were exposed to sunlight as the restriction movement forcing them to staying at home. Thus, it effects the SEL factors with Hb concentration in the study as they may have been less likely to venture outdoor and acquire sufficient sunlight. In a study carried out to examine the effect of COVID-19 on 25(OH)D levels among children living in Guangzhou, China found 25(OH)D levels tended to decrease gradually with age, and reduced exposure to sunlight has been found associated with lower 25(OH)D serum level in children 3 to 6 years of age (Yu et al., 2020). However, no study was found carried out among university students.

No significant association found could be also due to less body surface area exposed to sunlight. The majority of our subjects were Malay females, as a study has shown that girls who wear clothing with a headscarf cover have a higher risk of vitamin D insufficiency (Chin, Jamil & Norazirah, 2018). Duration to sun exposure was not associated with Hb could be due to sun protection behaviour as most of our participants were sunscreen when going outside (Nimitphong & Holick, 2013). The associations of dietary and supplementary vitamin D intakes with Hb concentration among participants is shown in **Figure 4.2** and **Figure 4.3**.

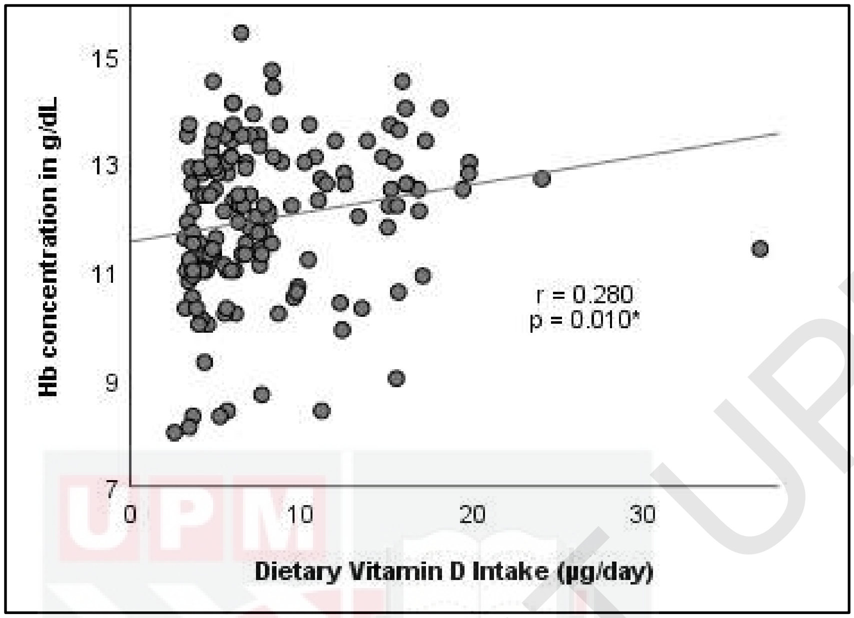


Figure 4.2 The association between dietary vitamin D intake and Hb concentration (g/dl)

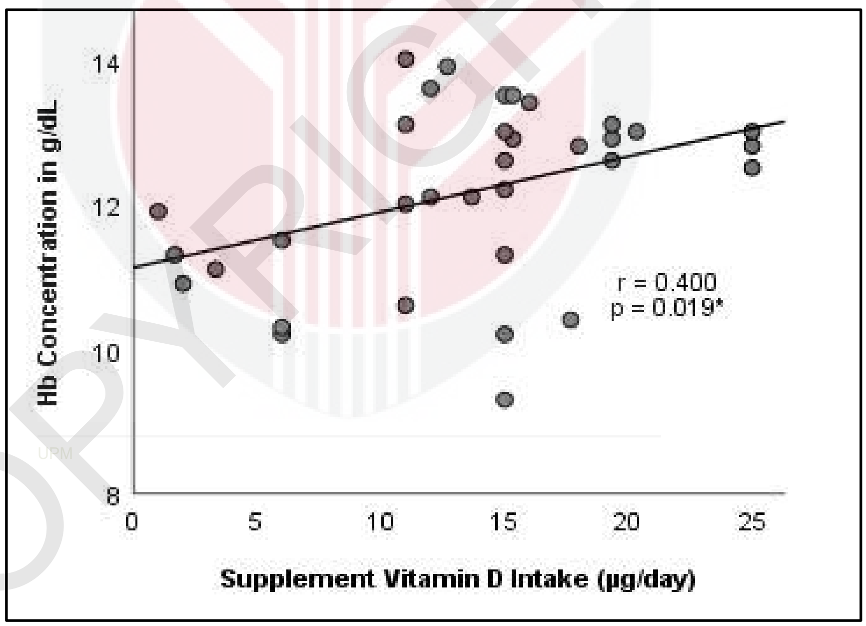


Figure 4.3 The association between supplement vitamin D usage and Hb concentration (g/dL)

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the prevalence of anaemia among participants was 38.7%, which was considered as severe public health problem, with a mean Hb of 12.10 ± 1.38 g/dL. The mean ± SD of dietary vitamin D intake was low (8.10 ± 5.08), with 85.2% of participants were reported to have intake below the RNI. From 14 food items assessed using FFLQ, the highest mean ± SD vitamin D intake was sardine (1.22 ± 1.56) followed by cod liver oil (1.16 ± 2.96) and mackerel (0.88 ± 1.30) in µg/day. In this study, participants' mean ± SD intake of supplemental vitamin D intake was below the RNI (13.56 ± 6.38 µg/day). SEL per week was 2.08 ± 1.11 hours with a BSA fraction of 0.30 ± 0.22 and SEI of 2.50 ± 1.46. The majority of participants had low SEI, which indicates low exposure to sunlight. The study demonstrated associations observed between both dietary vitamin D intake (r= 0.28, p= 0.010) and supplemental vitamin D intake (r= 0.400, p= 0.019) with Hb concentration. However, no significant association was observed between the SEL, BSA fraction and SEI with Hb concentration as the SEL and SEI were considered low overall.

An overall high prevalence of anaemia highlighted in the study suggests it is needed to develop appropriate nutrition interventions; vitamin D supplementation could be the immediate measure to cure anaemia, while food diversification and food fortification might be the long-term measures for the management of anaemia. The low exposure to sunlight indicates that female undergraduate students had spent less time outdoors than indoors, which may be because of MCO during the data collection period. The result also showed that the attitudes towards sun protection were adequate. Most participants had higher use of sunscreen and stayed under shade or used an umbrella as protection from sunlight. However, there is a need to plan an intervention program possibly to emphasize and promote sun exposure as the primary contributor to vitamin D status.

5.2 Limitations

The present study only focused on female undergraduates in Universiti Putra Malaysia, which did not include other female undergraduates from other universities. Therefore, the results cannot be taken as representative of all female university students in Malaysia. Secondly, this study is a cross-sectional study where the determination of causal association could not be identified due to this study design in which the exposure (vitamin D-related factors) and outcome (Hb concentration) were measures at the same time. Cross-sectional studies are also difficult to compare because of differences in the populations studied, such as age and country differences in biochemical indices and cut-offs used to define iron deficiency and varying methods of assessing diet. Besides, dietary and sun exposure assessments that have been carried out solely depended on participant's memories and honesty, causing under and over-reporting of dietary intake and SEL. Moreover, FFLQ, as one of the nutritional assessments for dietary and supplement intake of vitamin D, had a risk of under-reporting/over-reporting of food consumption and assessment of subway item in the FFLQ is not clear and did not specify into the specified subway as there are many types of subway is available at the market. Other factors that might affect Hb concentration, such as menstrual cycle and physical activity did not assess during the study. Since information regarding the entire diet was not gathered in the current study, it was not possible to analyse the dietary intake of other confounding variables such as iron, vitamin B12 and folate.

5.3 Recommendations

Several recommendations were recommended for future studies. Firstly, wider population and location which includes female students from other universities were needed as well so that the findings can be generalized to university students in Malaysian population. Next, it is recommended to use the food diary in order to record the consumption of dietary intake to reduce the risk of under or over reporting of nutrient intake. Further studies are also needed to understand more on the importance of dietary vitamin D intake as well to improve vitamin D status. Moreover, the present study suggested that university student particularly female student should consume rich vitamin D food and take supplementation if required to improve Hb concentration. Last but not least, university students and women of reproductive age are at an increased risk of iron inadequacy and associated complications. Therefore, a comprehensive combination of public health initiatives addressing iron shortage, anaemia, and vitamin D inadequacy is required.

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Appendix B: Information sheet and consent form (English/Malay)

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

FORM 2.4: RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

1. STUDY TITLE:

The association between omega-3 & vitamin D intake, sun exposure level, supplement usage and knowledge, attitude & practice (KAP) on IDA with haemoglobin concentration in female students of Universiti Putra Malaysia.

2. INTRODUCTION:

Anaemia is a condition where there is a lack of red blood cells (RBSs), which serves to carry oxygen in our body, to meet the basic needs of humans. The lack of RBCs may be determined by measuring one of the important iron status indicators, which is haemoglobin. Iron deficiency anaemia (IDA) was found to be the most significant contributor in anaemia. IDA is a condition where the balance between iron consumption from food, stores and loss from the body are not enough to support production of the RBCs. Findings from previous studies have suggested that dietary intake omega-3 & vitamin D intake, sun exposure level, supplement usage and knowledge, attitude & practice (KAP) may have an effect on how iron is absorbed and used in the body. Therefore, this study aims to determine the association between omega-3 & vitamin D intake, supplement usage, sun exposure level, and knowledge, attitude & practice (KAP) on IDA with haemoglobin concentration among female students in UPM. In this study, approximately 171 participants will be involved.

3. WHAT WILL YOU HAVE TO DO?

You will first be screened to assess your eligibility to take part in the study. Inclusion criteria includes Malaysian female undergraduate and postgraduate students, age between 18 to 49 years old with no history of chronic health conditions such as diabetes mellitus, metabolic disorder, chronic kidney disease and gastrointestinal related disorders, do not regularly consume nutritional supplements and not donated blood for the past 6 months. You will then be required to complete a set of questionnaires to obtain information on socio-demographic characteristics. Your body weight and height will be measured by the researcher in a closed room. Your habitual dietary intake over the past month will be assessed by recording your foods and beverages consumption through a food frequency questionnaire. You will be also required to complete another set of questionnaires on your supplement usage, general sun exposure level, knowledge, attitude and practice (KAP) on IDA. A small amount of blood from the finger prick will be collected using a small needle called lancet to assess the haemoglobin level. Estimated duration to complete the assessment will be 20-30 minutes.

4. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

Students who are male, non-Malaysian, pregnant, lactating and menopause women, individual who have celiac disease, gastroesophageal reflux disease, diabetes mellitus and metabolic disorder, individual that regularly consume nutritional supplements, engaged in excessive exercise (>4 hour/week) and have

JKEUPM/FORM 2.4
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donated blood for the past 6 months will be excluded from this study. You have been chosen because you satisfy the inclusion criteria for the study.

5. WHAT WILL BE THE BENEFITS OF THE STUDY:

a) TO YOU AS THE SUBJECT?

You will be provided with a summary of dietary analysis, body weight status (body weight, height, body mass index) and haemoglobin concentration data. Dietary analysis will be performed by the researcher on completion of the study using dietary analysis software. We will provide you information on your nutrient intake of omega-3, vitamin D and supplement usage. You also will know your total duration of sun exposure for a week and also your general KAP level on IDA.

b) TO THE INVESTIGATOR?

Findings of this study will provide information on sociodemographic characteristics, body weight status, omega-3 and vitamin D intake, supplement usage, sun exposure level and KAP level on IDA as well as haemoglobin concentration among female students in UPM. Findings of this study also will help nutritionist and health promotion planners to develop appropriate intervention and health promotion programs in improving overall nutritional and health status as well as to reduce anaemia cases in female students. Besides, this information will help to promote the dietary components used such as omega-3 and vitamin D among general population, which will later help to reduce the risk of developing anaemia.

6. WHAT ARE THE POSSIBLE RISKS?

This study has minimal risk where it involves finger prick method to obtain small amounts of blood, anthropometry measurements (body weight, and height) and also filling up questionnaires including omega-3 and vitamin D dietary intake, supplement usage, sun exposure level, and KAP level on IDA. If you were found to have low haemoglobin level after the measurement, you will be advised to seek further medical attention from your general practitioner.

7. WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?
All information which is collected about you during this course of the research will be kept strictly confidential and anonymised so that only the researcher carrying out the research will have access to such information. The researcher will also not provide any participant information to any party without the consent of subjects.

8. WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

If you have any enquiries, you can contact as follows:

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Supervisor
Dr Salma Faeza Binti Ahmad Fuzi
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*Please initial here if you have read and understood the contents of this page*_______

9. CONSENT

I .... Identity Card No. ....

address..

....hereby voluntarily agree to take part in the research stated above *(clinical /drug trial/video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature .... Signature ....
(Respondent) (Witness)

Date :.. Name :..

I/C No. :..

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date .... Signature ....
(Researcher)

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

BORANG 2.4: PENERANGAN DAN PERSETUJUAN RESPONDEN

Sila baca maklumat berikut dengan teliti. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

1. TAJUK KAJIAN

Hubungan antara pengambilan omega-3 & vitamin D, penggunaan supplemen, tahap pendedahan cahaya matahari, dan pengetahuan, sikap & amalan (KAP) mengenai anemia kekurangan zat besi dengan kepekatan hemoglobin dalam kalangan pelajar wanita Universiti Putra Malaysia.

2. PENGENALAN

Anemia adalah satu keadaan di mana terdapat kekurangan sel darah merah, yang berfungsi untuk membawa oksigen ke dalam badan kita bagi memenuhi keperluan asas manusia. Kekurangan sel darah merah boleh ditentukan dengan mengukur salah satu penunjuk penting bagi status zat besi iaitu hemoglobin. Anemia kekurangan zat besi (IDA) didapati sebagai penyumbang paling utama dalam anemia. Anemia zat besi merupakan suatu keadaan di mana keseimbangan antara pengambilan zat besi dalam makanan, simpanan dan kehilangan daripada tubuh tidak mencukupi untuk menyokong pengeluaran sel darah merah. Penemuan dari kajian terdahulu menunjukkan bahawa pengambilan makanan omega-3 & pengambilan vitamin D, penggunaan supplemen, tahap pendedahan cahaya matahari, dan pengetahuan, sikap & amalan (KAP) boleh memberi kesan kepada bagaimana zat besi diserap dan digunakan dalam tubuh. Oleh itu, kajian ini bertujuan untuk menentukan perkaitan antara pengambilan omega-3 & vitamin D, penggunaan supplemen, tahap pendedahan cahaya matahari, dan pengetahuan, sikap & amalan (KAP) mengenai anemia kekurangan zat besi dengan kepekatan hemoglobin dalam kalangan pelajar wanita Universiti Putra Malaysia. Anggaran seramai 171 pelajar akan terlibat dalam kajian ini.

3. APAKAH YANG PERLU ANDA LAKUKAN?

Anda akan menjalani pemeriksaan untuk menilai kelayakan anda untuk mengambil bahagian dalam kajian ini. Kriteria inklusi termasuk staf dan pelajar wanita, berumur antara 19 hingga 49 tahun tanpa sejarah gangguan gastrousus dan penyakit kronik seperti kencing manis dan penyakit metabolisma, tidak mengambil suplemen nutrisi dengan kerap dan tidak menderma darah selama 6 bulan yang lalu. Anda kemudian akan dikehendaki untuk melengkapkan satu set soal selidik untuk mendapatkan maklumat mengenai ciri sosio-demografi. Berat dan ketinggian badan anda akan diukur oleh penyelidik di dalam bilik tertutup. Pengambilan makanan anda bagi sebulan yang lepas akan dinilai melalui borang kekerapan pengambilan makanan. Anda juga diminta untuk melengkapkan satu borang berkaitan penggunaan supplemen, pendedahan kepada cahaya matahari, pengetahuan, sikap dan amalan terhadap anemia kekurangan zat besi. Sebelingan kecil darah dari jari telunjuk akan dikumpulkan menggunakan jarum kecil dikenali sebagai lancet untuk menguji tahap hemoglobin. Anggaran masa 20-30 minit bagi melengkapkan proses pengambilan data.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

Pelajar lelaki, bukan warganegara Malaysia, hamil, menyusu dan menopaus, individu yang mempunyai penyakit celiac, penyakit refluks gastroesofagus, diabetes mellitus dan gangguan metabolik, individu yang selalu mengambil suplemen makanan, melakukan senaman berlebihan (> 4 jam/minggu) dan mempunyai sejarah menderma darah darah dalam tempoh 6 bulan terakhir akan dikeluarkan dari kajian ini. Anda telah dipilih kerana anda memenuhi kriteria penyertaan untuk kajian ini.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Anda akan diberikan ringkasan analisis diet, status berat badan (berat badan, tinggi badan, indeks jisim badan) dan data kepekatan hemoglobin. Analisis diet akan dilakukan oleh pengkaji setelah menyelesaikan kajian menggunakan perisian analisis diet. Kami akan memberi anda maklumat mengenai pengambilan nutrien omega-3, vitamin D dan penggunaan supplemen anda. Anda juga akan mengetahui jangka masa pendedahan cahaya matahari selama seminggu dan juga tahap KAP umum anda pada anemia kekurangan zat besi.

b) KEPADA PENYELIDIK?

Penemuan kajian ini akan memberi maklumat tentang ciri sosio-demografi, status berat badan, pengambilan omega-3 dan vitamin D, penggunaan supplemen, tahap pendedahan cahaya matahari dan tahap KAP pada IDA serta kepekatan hemoglobin pada pelajar perempuan di Universiti Putra Malaysia. Penemuan kajian ini juga akan membantu perancang pemakanan dan promosi kesihatan untuk mengembangkan program intervensi dan promosi kesihatan yang sesuai dalam meningkatkan status pemakanan dan kesihatan secara keseluruhan serta mengurangkan kes anemia pada pelajar wanita. Selain itu, maklumat ini akan membantu mempromosikan komponen makanan yang digunakan seperti omega-3 dan vitamin D di kalangan penduduk umum, yang kemudian akan membantu dalam mengurangkan risiko menghidapi anemia.

6. ADAKAH IA BERISIKO?

Kajian ini mempunyai risiko minimum di mana ia melibatkan kaedah "*finger prick*" untuk memperoleh sedikit darah, pengukuran antropometri (berat badan, dan tinggi) dan juga mengisi soal selidik mengenai pengambilan makanan omega-3 dan vitamin D, penggunaan supplemen, tahap pendedahan kepada cahaya matahari dan tahap KAP pada anemia kekurangan zat besi. Sekiranya anda didapati mempunyai tahap hemoglobin yang rendah selepas analisis darah dilakukan, anda akan dinasihatkan untuk mendapatkan rawatan lanjut dari pakar perubatan.

7. ADAKAH MAKLUMAT DAN IDENTITI SAYA KEKAL RAHSIA?

Semua maklumat yang dikumpulkan mengenai anda semasa kursus ini akan disimpan secara rahsia dan tidak dikenali supaya hanya penyelidik yang menjalankan penyelidikan untuk mengakses maklumat tersebut. Penyelidik juga tidak akan memberikan sebarang maklumat peserta kepada mana-mana pihak tanpa persetujuan peserta.

8. SIAPA YANG SAYA PERLU HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEMASA MENGIKUTI PENYELIDIKAN INI?

Sekiranya ada mempunyai sebarang pertanyaan, anda boleh menghubungi seperti berikut:

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Nurul Aqilah bt Yunos
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Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini ________

9. PERSETUJUAN

Saya…………………………………………… No Kad Pengenalan. …………………………… beralamat……………………………………………………………………………………………………… ……………………….dengan ini bersetuju untuk mengambil bahagian secara sukarela dalam penyelidikan yang tersebut di atas *(kajian klinikal/percubaan ubat-ubatan/rakaman video/kumpulan sasaran/temuduga/ soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti tertulis pada Helaian Penerangan Responden). Saya memahami bahawa saya berhak menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan.Saya juga memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

Saya* berminat / tidak berminat untuk mengetahui keputusan kajian yang melibatkan saya.

I setuju/tidak bersetuju untuk imej/gambar/rakaman video/ rakaman suara digunakan dalam apa jua bentuk penerbitan atau pembentangan. (sekiranya berkaitan).

*potong yang tidak berkenaan

Tandatangan ……………………………… (*Responden*)

Tarikh :……………………………………..

Tandatangan ……………………………… (*Saksi*)

Nama :……………………………………..

No. K/P: ……………………………………..

Saya mengesahkan bahawa saya telah menerangkan kepada responden ini sifat dan tujuan penyelidikan yang tersebut di atas.

Tarikh ……………………………….

Tandatangan ……………………………… (*Penyelidik*)

Appendix F: Set of questionnaires

Reference No. / *No. Rujukan:*

ASSOCIATION BETWEEN OMEGA-3 & VITAMIN D INTAKE, SUPPLEMENT CONSUMPTION, SUN EXPOSURE LEVEL, AND KNOWLEDGE, ATTITUDE & PRACTICE (KAP) ON IDA WITH HAEMOGLOBIN CONCENTRATION IN FEMALE STUDENTS OF UNIVERSITI PUTRA MALAYSIA.

Hubungan antara pengambilan omega-3 & vitamin D, pengambilan supplemen, tahap pendedahan cahaya matahari, dan pengetahuan, sikap & amalan (KAP) mengenai anemia kekurangan zat besi dengan kepekatan hemoglobin dalam kalangan pelajar wanita Universiti Putra Malaysia.

Questionnaire/ *Borang Soal Selidik*

All of your information is for research purposes and will be kept confidential. Please follow the instructions and answer all of the questions. Thank you for your participation.

Segala maklumat anda berikan adalah untuk tujuan penyelidikan sahaja dan ia tidak akan didedahkan kepada mana-mana pihak. Kami berharap anda dapat memberi kerjasama penuh dan jujur dalam menjawab setiap soalan. Terima kasih atas kerjasama anda.

Date of collection / *Tarikh pengambilan* : ___ / ___ / _______

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM) UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

FORM 2.4: RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

STUDY TITLE:

The association between omega-3 & vitamin D intake, sun exposure level, supplement usage and knowledge, attitude & practice (KAP) on IDA with haemoglobin concentration in female students of Universiti Putra Malaysia.

INTRODUCTION:

Anaemia is a condition where there is a lack of red blood cells (RBSs), which serves to carry oxygen in our body, to meet the basic needs of humans. The lack of RBCs may be determined by measuring one of the important iron status indicators, which is haemoglobin. Iron deficiency anaemia (IDA) was found to be the most significant contributor in anaemia. IDA is a condition where the balance between iron consumption from food, stores and loss from the body are not enough to support production of the RBCs. Findings from previous studies have suggested that dietary intake omega-3 & vitamin D intake, sun exposure level, supplement usage and knowledge, attitude & practice (KAP) may have an effect on how iron is absorbed and used in the body. Therefore, this study aims to determine the association between omega-3 & vitamin D intake, supplement usage, sun exposure level, and knowledge, attitude & practice (KAP) on IDA with haemoglobin concentration among female students in UPM. In this study, approximately 171 participants will be involved.

1.WHAT WILL YOU HAVE TO DO?

You will first be screened to assess your eligibility to take part in the study. Inclusion criteria includes Malaysian female undergraduate and postgraduate students, age between 18 to 49 years old with no history of chronic health conditions such as diabetes mellitus, metabolic disorder, chronic kidney disease and gastrointestinal related disorders, do not regularly consume nutritional supplements and not donated blood for the past 6 months. You will then be required to complete a set of questionnaires to obtain information on socio-demographic characteristics. Your body weight and height will be measured by the researcher in a closed room. Your habitual dietary intake over the past month will be assessed by recording your foods and beverages consumption through a food frequency questionnaire. You will be also required to complete another set of questionnaires on your supplement usage, general sun exposure level, knowledge, attitude and practice (KAP) on IDA. A small amount of blood from the finger prick will be collected using a small needle called lancet to assess the haemoglobin level. Estimated duration to complete the assessment will be 20-30 minutes.

2.WHO SHOULD NOT PARTICIPATE IN THE STUDY?

Students who are male, non-Malaysian, pregnant, lactating and menopause women, individual who have celiac disease, gastroesophageal reflux disease, diabetes mellitus and metabolic disorder, individual that regularly consume nutritional supplements, engaged in excessive exercise (>4 hour/week) and have donated blood for the past 6 months will be excluded from this study. You have been chosen because you satisfy the inclusion criteria for the study.

3.WHAT WILL BE THE BENEFITS OF THE STUDY:

a) TO YOU AS THE SUBJECT?

You will be provided with a summary of dietary analysis, body weight status (body weight, height, body mass index) and haemoglobin concentration data. Dietary analysis will be performed by the researcher on completion of the study using dietary analysis software. We will provide you information on your nutrient intake of omega-3, vitamin D and supplement usage. You also will know your total duration of sun exposure for a week and also your general KAP level on IDA.

b) TO THE INVESTIGATOR?

Findings of this study will provide information on sociodemographic characteristics, body weight status, omega-3 and vitamin D intake, supplement usage, sun exposure level and KAP level on IDA as well as haemoglobin concentration among female students in UPM. Findings of this study also will help nutritionist and health promotion planners to develop appropriate intervention and health promotion programs in improving overall nutritional and health status as well as to reduce anaemia cases in female students. Besides, this information will help to promote the dietary components used such as omega-3 and vitamin D among general population, which will later help to reduce the risk of developing anaemia.

4.WHAT ARE THE POSSIBLE RISKS?

This study has minimal risk where it involves finger prick method to obtain small amounts of blood, anthropometry measurements (body weight, and height) and also filling up questionnaires regarding omega-3 and vitamin D dietary intake, supplement usage, sun exposure level, and KAP level on IDA. If you were found to have low haemoglobin level after the measurement, you will be advised to seek further medical attention from your general practitioner.

5.WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

All information which is collected about you during this course of the research will be kept strictly confidential and anonymised so that only the researcher carrying out the research will have access to such information. The researcher will also not provide any participant information to any party without the consent of subjects.

6.WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

If you have any enquiries, you can contact as follows:

Researcher:	Supervisor
Tanusha Devi A/P Santhrasagar	Dr Salma Faeza Binti Ahmad Fuzi
011-16393512 / tanushadevi17@gmail.com	03-86092974 / salmafaeza@upm.edu.my

Nurul Izzah Abdullah
013-2036191 / izzahnoradlin@gmail.com

Nurul Aqilah bt Yunos
019-2336768/ aqilah.yarinos@gmail.com

*Please initial here if you have read and understood the contents of this page*____

2. CONSENT

I …………………………………… Identity Card No.……………………………
address……………………………………………………………………………………………
…………...

………………………………………………….. hereby voluntarily agree to take part in the research stated above *(clinical /drug trial/video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse

effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature ……..…………………………… Signature ……..……………………………
(Respondent) (Witness)

Date :……………………………………….. Name :……………………………………..

I/C No. :……………………………………..

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date ……..………………………… Signature ……..…………………………..(Researcher)

SECTION A: SOCIO-DEMOGRAPHIC CHARACTERISTICS / *BAHAGIAN A: CIRI SOSIODEMOGRAFI*

Instructions: Below is a list of questions on your socio-demographic characteristics. Please fill in the following details.

***Arahan: Berikut merupakan senarai soalan yang berkaitan dengan ciri sosio-demografi anda. Sila isi butiran berikut*.**

1. Date of birth : ___ / ___ / _______(dd/mm/yyyy)

   Tarikh lahir : ___ / ___ / _______ *(tt/bb/tttt)*

2. Age : __________ years old

   Umur : __________ *tahun*

3. Ethnicity : [ ] Malay / *Melayu*

   Etnik
   [ ] Chinese / *Cina*
   [ ] Indian / *India*
   [ ] Others / *Lain-lain*

4. Faculty of study : _______________________________________

   Fakulti pengajian

5. Course of study : _______________________________________

   Program pengajian

6. Year of study : [ ] 1st Year / *Tahun 1*

   Tahun pengajian
   [ ] 2nd Year / *Tahun 2*
   [ ] 3rd Year / *Tahun 3*
   [ ] 4th Year / *Tahun 4*
   [ ] 5th Year / *Tahun 5*

7. Monthly allowance : _______________________________________

   Elaun bulanan

8. Are you from a nutritional/dietetics background?

	Adakah anda dari latar belakang pemakanan / dietetik?	( ) Yes/ *Ya* ( ) No/ *Tidak*
9.	Do you have illnesses such as celiac disease, gastroesophageal reflux disease, diabetes mellitus and metabolic disorder? *Adakah anda mempunyai masalah kesihatan seperti penyakit celiac, penyakit refluks gastroesofagus, diabetes mellitus dan gangguan metabolik*	( ) Yes/ *Ya* State/*nyatakan*_______________ ( ) No/ *Tidak*
10.	Do you take any regular supplements? *Adakah anda mengambil sebarang suplemen secara berkala?*	( ) Yes/ *Ya* State/*nyatakan*_______________ ( ) No/ *Tidak*
11.	For the past 6 months, have you donated your blood? *Adakah anda ada menderma darah dalam tempoh 6 bulan yang lepas?*	( ) Yes/ *Ya* ( ) No/ *Tidak*
12.	Have you engaged in any excessive exercise (> 4 h/week)? *Adakah anda ada melakukan senaman berlebihan (> 4 jam / minggu)*	( ) Yes/ *Ya* ( ) No/ *Tidak*
13.	Are you on your 1st-7th day of heavy menstruation? *Adakah anda sedang kedatangan haid pada hari pertama hingga ketujuh?*	( ) Yes/ *Ya* ( ) No/ *Tidak*
14.	Are you a vegetarian? *Adakah anda seorang vegetarian?*	( ) Yes/ *Ya* ( ) No/ *Tidak*

SECTION B: ANTHROPOMETRIC MEASUREMENT/ *BAHAGIAN B: PENGUKURAN ANTHROPOMETRIK*

Instruction: This section will be filled by the researchers. / *Arahan: Bahagian ini akan diisi oleh penyelidik.*

Measurement / ***Pengukuran***	**Reading 1 /** ***Bacaan 1***	**Reading 2 /** ***Bacaan 2***	**Average /**
Weight / ***Berat*** **(kg)**			
Height / ***Tinggi*** **(m)**			
BMI / ***BMI*** **(kg/m²)**			

SECTION C: HAEMOGLOBIN MEASUREMENT / ***BAHAGIAN C: PENGUKURAN HEMOGLOBIN***

Instruction: This section will be filled by the researchers. / ***Arahan: Bahagian ini akan diisi oleh penyelidik.***

Measurement / ***Bacaan***	**Reading 1 /** ***Bacaan 1***	**Reading 2 /** ***Bacaan 2***	**Average**
Haemoglobin level / ***Paras hemoglobin*** **(g/dL)**			

SECTION D: DIETARY INTAKE / *BAHAGIAN D: PENGAMBILAN DIET*

Instructions: This section will cover 2 dietary intakes, Omega-3 which is D(I) and Vitamin D will be D(II). Tick (/) on the space provided based on your dietary intake in a month. For each food listed, you are only required to tick 1 box. Please refer to the printed visuals to estimate usual serving size. / *Arahan: Bahagian ini merangkumi 2 pengambilan makanan, Omega-3 iaitu D (I) dan Vitamin D iaitu D (II). Tandakan (/) pada ruang yang disediakan berdasarkan pengambilan makanan anda dalam sebulan. Untuk setiap makanan yang disenaraikan, anda hanya perlu menandakan 1 kotak. Sila rujuk gambaran bercetak untuk mengganggar saiz sajian.*

D(I): OMEGA-3 INTAKE/ *D(I): PENGAMBILAN OMEGA-3*

Food items ***Jenis makanan***	**Serving size** ***Saiz hidangan***	**Frequency *Frekuensi***					
		≥ 1 time/day ***≥ sekali sehari***	**4–6 times/week** ***4-6 kali/minggu***	**1–3 times/week** ***1-3 kali/minggu***	**2–3 times/month** ***2-3 kali/bulan***	**Once a month or less** ***Sekali/bulan, jarang***	**Never** **Tidak pernah**
Fish and seafood **Ikan dan makanan laut**							
Anchovy *Ikan bilis*	1/3 Cup *1/3 Cawan*						
African bream *Ikan tilapia*	1 Piece *1 Ekor*						
Carp *Ikan Kap*	1 Piece *1 Ekor*						
Crab	1 Piece						

Ketam	*1 Ekor*						
Snakehead fish *Ikan* *Haruan*	1 Piece *1 Ekor*						
Eel Belut	1 Piece 1 Ekor						

Food items *Jenis makanan*	Serving size *Saiz hidangan*	Frequency/ *Frekuensi*					
		≥ 1 time/day ***≥ sekali sehari***	**4–6 times/week** ***4-6 kali/minggu***	**1–3 times/week** ***1-3 kali/minggu***	**2–3 times/month** ***2-3 kali/bulan***	**Once a month, or less** ***Sekali/bulan, jarang***	**Never** ***Tidak pernah***
Sea bass *Ikan* *Siakap*	1 Piece *1 Ekor*						
Sardine *Sardine*	1 Piece *1 Ekor*						
Tuna *Tuna/Tongkol*	1 Piece *1 Ekor*						
Salmon *Salmon*	1 Piece *1 Ekor*						
Sultan fish *Jelawat*	1 Piece *1 Ekor*						
Spanish mackerel *Ikan Tenggiri*	1 Piece *1 Ekor*						
Oyster	1 Piece						

Tiram	1 Ekor						
Black pomfret *Bawal hitam*	1 Piece *1 Ekor*						
Stingray *Ikan Pari*	1 Piece *1 Ekor*						

Food items ***Jenis makanan***	**Serving size** ***Saiz hidangan***	**Frequency/ *Frekuensi***					
		≥ 1 time/day **≥ *sekali sehari***	**4–6 times/week** ***4-6 kali/minggu***	**1–3 times/week** ***1-3 kali/minggu***	**2–3 times/month** ***2-3 kali/bulan***	**Once a month or less** ***Sekali/bulan, jarang***	**Never** ***Tidak pernah***
Walnut oil *Minyak walnut*	1 Tea spoon *1 Sudu teh*						
Flaxseed oil *Minyak flaxseed*	1 Tea spoon *1 Sudu teh*						
Margerine *marjerin*	1 Tea spoon *1 Sudu teh*						
Vegetables **Sayur-sayuran**							
Soya bean milk *Susu kacang soya*	1 Glass *1 Gelas*						
Soya bean curd (slices) *Fucok*	1 Slice *1 Keping*						
Soya bean curd *Tau-hoo*	1 Slice *1 Keping*						
Bean sprout *Taugeh*	1 Cup *1 Cawan*						

Food items ***Jenis makanan***	**Serving size** ***Saiz hidangan***	**Frequency/ *Frekuensi***					
		≥ 1 time/day ***≥ sekali sehari***	**4–6 times/week** ***4-6 kali/minggu***	**1–3 times/week** ***1-3 kali/minggu***	**2–3 times/month** ***2-3 kali/bulan***	**Once a month or less** ***Sekali/bulan, jarang***	**Never** ***Tidak pernah***
Silver pomfret *Bawal putih*	1 Piece *1 Ekor*						
Shrimp *udang*	1 Medium size *1 Ekor sederhana*						
Plant-based oil **Minyak sayuran**							
Palm olein oil *Minyak sawit*	1 Tea spoon *1 Sudu teh*						
Soya bean oil *Minyak kacang soya*	1 Tea spoon *1 Sudu teh*						
Perilla oil *Minyak perilla*	1 Tea spoon *1 Sudu teh*						
Cod oil Minyak kod	1 Tea spoon *1 Sudu teh*						
Canola oil *Minyak kanola*	1 Tea spoon *1 Sudu teh*						

Food items *Jenis makanan*	Serving size *Saiz hidangan*	Frequenc/ *Frekuensi*					
		≥ 1 time/day **≥ *sekali sehari***	**4–6 times/week** ***4-6 kali/minggu***	**1–3 times/week** ***1-3 kali/minggu***	**2–3 times/month** ***2-3 kali/bulan***	**Once a month or less** ***Sekali/bulan, jarang***	**Never** ***Tidak pernah***
Green leavy vegetables *Sayur berdaun hijau*	1 Cup *1 Cawan*						
Fermented soy bean *Tempeh*	1 Slice *1 Keping*						
Pea *Kacang*	1 Cup *1 Cawan*						
Meat, poultry and eggs **Daging dan telur**							
Hen egg (omega-3) Telur (omega-3)	1 Whole 1 Biji						
Pork Daging khinzir	1 Piece 1 Keping						
Beef Daging lembu	1 Piece 1 Keping						
Lamb Daging kambing	1 Piece 1 Keping						

Chicken Ayam	1 Piece 1 Ketul						

Food items *Jenis makanan*	Serving size *Saiz hidangan*	Frequency/ *Frekuensi*					
		≥ 1 time/day **≥ *sekali sehari***	**4–6 times/week** ***4-6 kali/minggu***	**1–3 times/week** ***1-3 kali/minggu***	**2–3 times/month** ***2-3 kali/bulan***	**Once a month, less or never** ***Sekali/bulan, jarang***	**Never** ***Tidak pernah***
Milk and dairy products **Susu dan hasil tenusu**							
Butter *Mentega/krim*	1 Tea spoon *1 Sudu teh*						
Milk (omega-3) *Susu (omega-3)*	1 Glass *1 Gelas*						
Full cream milk *Susu rendah lemak*	1 Glass *1 Gelas*						
Skimmed milk *Susu skim*	1 Dessert spoon *1 Sudu makan*						
Low fat milk *Susu rendah lemak*	1 Glass *1 Gelas*						

D(II): VITAMIN D INTAKE/ *D(II): PENGAMBILAN VITAMIN D*

Food Consume / *Pengambilan Makanan*	**Never or <1 per mo / *Tidak pernah atau <1 setiap bulan***	**1-3 per month / *1 - 3 setiap bulan***	**1 per week / *1 kali seminggu***	**2-4 per week / *2-4 kali seminggu***	**5-6 per week / *5 -6 kali seminggu***	**1 per day / *1 hari sekali***	**4-5 per day / *4- 5 kali sehari***	**6+ per day / *6 atau lebih kali sehari***
1. Milk (1 Cup) / *Susu (1 cawan)*								
2. Soymilk (1 Cup) / *Susu soya (1 cawan)*								
3. Cereal (3/4 Cup) / *bijirin (¾ cawan)*								
4. Subway Sandwich (6 Inch) / *Roti Sandwich (6 inci)*								
5. Margarine (1 Tsp) / *Marjerin (1 sd teh)*								
6. Orange Juice (1 Cup) / *Jus Oren (1 Cawan)*								
7. Cooked Chicken Liver (3 ½ Oz or 100g) / *Hati ayam (3 ½ auns)*								
8. Egg (1 Whole) / *Telur (Sebiji)*								
9. Codliver Oil (1 Tsp) / *Minyak ikan kod (1 sd teh)*								
10. Salmon (3 ½ Oz or 100g) / *Ikan salmon (3 ½ auns)*								

11. Mackerel (3 ½ Oz or 100g) / *Ikan tenggiri (3 ½ auns)*								
12. Sardine (3 ½ Oz or 100g) / *IKan Sardin (3 ½ auns)*								
13. Eel, Cooked (3 ½ Oz or 100g) / *Belut dimasak (3 ½ auns)*								
14. Tuna (3 ½ Oz or 100g) / *Ikan tuna (3 ½ auns)*								

<u>SECTION E: SUPPLEMENT INTAKE / *BAHAGIAN E: PENGAMBILAN SUPPLEMENT*</u>

Instructions: Tick (/) the box on how often you (on average) have used the amount specified and write the amount of supplement that you have consumed. / *Arahan: Tandakan (/) pada seberapa kerap anda (rata-rata) telah menggunakan jumlah yang ditentukan dan tulis jumlah makanan tambahan yang telah anda habiskan.*

Supplement consumed / *Supplemen dimakan*	**Never or <1 per mo / *Tidak pernah atau <1 setiap bulan***	**1-3 per month / *1 - 3 setiap bulan***	**1 per week / *1 kali seminggu***	**2-4 per week / *2-4 kali seminggu***	**5-6 per week /*5 -6 kali seminggu***	**1 per day / *1 hari sekali***	**2-3 per day / *2-3 kali sehari***	**4-5 per day / *4- 5 kali sehari***	**6+ per day / *6 atau lebih kali sehari***
Multiple Vitamin (list amount__) / *Pelbagai vitamin (jumlah senarai__)*									
Vitamin D (list amount__) / *Vitamin D* *(jumlah senarai__)*									

Calcium Vitamin (list amount__) / *Vitamin kalsium* *(jumlah senarai__)*									
Calcium + Vit D (list amount__) / *Kalsium + Vit D (jumlah senarai__)*									
If you take any of the above, please list which you use, brand name etc: *Sekiranya anda mengambil salah satu perkara di atas, sila senaraikan yang anda gunakan, nama jenama dll:*									

SECTION F: SUN EXPOSURE LEVEL / *BAHAGIAN F: TAHAP PENDEDAHAN MATAHARI*

F(I). Instructions: Write how many minutes in the table given based on your time spent outdoors. / *F(I). Arahan: Tulis berapa minit dalam jadual yang diberikan berdasarkan masa yang anda habiskan di luar rumah.*

	7-9am /*7 -9 pagi*	**9-11am / *9 - 11 pagi***	**11am-1pm / 11 *pagi - 1 tengah hari***	**1-3pm / *1 -3 petang***	**3-5 pm / *3 -5 petang***	**5-7 pm / *5 -7 petang***
How much time did you spend outdoors between these periods? / *Berapa banyak masa yang anda habiskan di luar rumah dalam tempoh tersebut?*						

F(II). Instructions: Fill the number of days you go out in a week (0-7 days). / *F(II). Arahan: Isi bilangan hari anda keluar dalam masa seminggu (0-7 hari).*

	Days / *Hari*
In a week, how many days, you go out? / *Dalam seminggu, berapa hari, anda keluar?*	

F(III). Instructions: Circle one or more (1-7) against the exposed body area to sunlight which is applicable to you for each time period. / *F(III). Arahan: Bulatkan satu atau lebih (1-7) bagi kawasan tubuh badan anda yang terdedah kepada cahaya matahari bagi setiap masa yang dinyatakan.*

Time / *Masa*	**What areas of your body were exposed to sunlight? / *Kawasan tubuh badan anda yang terdedah kepada cahaya matahari?***
7-9am / *7-9 pagi*	(1) Face/*Muka* (2) Hands/*Tangan* (3) Full arms/*Seluruh lengan* (4) Half arms/*Separuh lengan* (5) Full legs/*Seluruh Kaki* (6) Half legs/*Separuh kaki* (7) Other (please specify)/*Lain-lain (sila nyatakan)* _________
9-11am / *9-11 pagi*	(1) Face/*Muka* (2) Hands/*Tangan* (3) Full arms/*Seluruh lengan* (4) Half arms/*Separuh lengan* (5) Full legs/*Seluruh Kaki* (6) Half legs/*Separuh kaki* (7) Other (please specify)/*Lain-lain (sila nyatakan)* _________
11am-1pm / *11 pagi - 1 tengah hari*	(1) Face/*Muka* (2) Hands/*Tangan* (3) Full arms/*Seluruh lengan* (4) Half arms/*Separuh lengan* (5) Full legs/*Seluruh Kaki* (6) Half legs/*Separuh kaki* (7) Other (please specify)/*Lain-lain (sila nyatakan)* _________
1-3pm / *1-3 petang*	(1) Face/*Muka* (2) Hands/*Tangan* (3) Full arms/*Seluruh lengan* (4) Half arms/*Separuh lengan* (5) Full legs/*Seluruh Kaki* (6) Half legs/*Separuh kaki* (7) Other (please specify)/*Lain-lain (sila nyatakan)* _________
3-5pm / *3-5 petang*	(1) Face/*Muka* (2) Hands/*Tangan* (3) Full arms/*Seluruh lengan* (4) Half arms/*Separuh lengan* (5) Full legs/*Seluruh Kaki* (6) Half legs/*Separuh kaki* (7) Other (please specify)/*Lain-lain (sila nyatakan)* _________
5-7pm / *5-7 petang*	(1) Face/*Muka* (2) Hands/*Tangan* (3) Full arms/*Seluruh lengan* (4) Half arms/*Separuh lengan* (5) Full legs/*Seluruh Kaki* (6) Half legs/*Separuh kaki* (7) Other (please specify)/*Lain-lain (sila nyatakan)* _________

F(IV). Instructions: For the following questions, think about what you do when you are outside during a sunny day. / *F(IV). Arahan: Untuk soalan berikut, fikirkan apa yang anda lakukan semasa berada di luar semasa hari yang cerah.*

	Never / *Tidak Pernah*	**Sometimes / *Kadang Kala***	**Always / *Selalu***
a) How often do you wear sunscreen / *Berapa kerap anda memakai pelindung matahari*			

b) How often do you stay in the shade or under an umbrella? / *Berapa kerap anda tinggal di bawah tempat teduh atau di bawah payung?*			

SECTION G: KNOWLEDGE, ATTITUDE & PRACTICE LEVEL / *BAHAGIAN G: TAHAP PENGETAHUAN, SIKAP & AMALAN*

G(I): Knowledge level / *G(I): Tahap pengetahuan*

Instructions: Below is a list of statements dealing with your general knowledge about IDA. Please indicate the correct answer for each statement. / *Arahan: Berikut adalah senarai pernyataan yang berkaitan dengan pengetahuan umum anda mengenai anemia kekurangan zat besi. Sila nyatakan jawapan yang betul untuk setiap pernyataan.*

Statement ***Pernyataan***	**Answers** ***Jawapan***
1. Have you heard about iron-deficiency anaemia? *Adakah anda pernah mendengar mengenai anemia kekurangan zat besi?*	a. Yes ( ) / *Ya ( )* b. No ( ) / *Tidak ( )* c. Don't know ( ) / *Tidak tahu ( )*
If yes in Question 1, i. Can you tell me how you can recognize someone who has anemia? *i. Bolehkah anda memberitahu saya bagaimana anda dapat mengenali seseorang yang mengalami anemia?*	a. Less energy/weakness ( ) / *Kekurang tenaga / kelemahan ( )* b. Paleness/pallor ( ) / *Kepucatan ( )* c. Spoon nails/bent nails (koilonychia) ( ) / *kuku bengkok ( )* d. More likely to become sick (less immunity to infections) ( ) / *Lebih cenderung menjadi sakit (kurang imuniti terhadap jangkitan) ( )* e. Other ( ) / *Lain-lain ( )* f. Don't know ( ) / *Tidak tahu ( )*
2. What are the health risks for infants and young children of a lack of iron in the diet? *Apakah risiko kesihatan terhadap bayi dan anak kecil yang kekurangan zat besi dalam makanan?*	a. Delay of mental and physical development ( ) / *Perkembangan mental dan fizikal yang lambat ( )* b. Other ( ) / *Lain-lain ( )* c. Don't know ( ) / *Tidak tahu ( )*
3. What are the health risks for pregnant women of a lack of iron in the diet? *Apakah risiko kesihatan terhadap wanita hamil yang kekurangan zat besi dalam makanan?*	a. Risk of dying during or after pregnancy ( ) / *Berisiko untuk meninggal dunia semasa atau selepas kehamilan ( )* b. Difficult delivery ( ) / *Kerumitan semasa bersalin ( )* c. Other ( ) / *Lain-lain ( )*

	d. Don't know ( ) / *Tidak tahu ( )*
4. What causes anaemia? *Apakah yang menyebabkan anemia?*	a. Lack of iron in the diet/eat too little, not much ( ) / *Kekurangan zat besi dalam makanan / makan terlalu sedikit, tidak banyak ( )* b. Sickness/infection (malaria, hookworm infection, other infection such as HIV/AIDS) ( ) / *Penyakit/jangkitan (malaria, jangkitan cacing tambang, jangkitan lain seperti HIV/AIDS) ( )* c. Heavy bleeding during menstruation ( ) / *Pendarahan yang terlampau semasa haid ( )* d. Other ( ) / *Lain-lain ( )* e. Don't know ( ) / *Tidak tahu ( )*
5. How can anaemia be prevented? *Bagaimana anemia dapat dicegah?*	a. Eat/feed iron-rich foods/having a diet rich in iron ( ) / *Makan/beri makanan kaya zat besi / diet yang kaya dengan zat besi ( )* b. Eat/give vitamin-C-rich foods during or right after meals ( ) / *Makan/beri makanan kaya vitamin C semasa atau selepas makan ( )* c. Take/give iron supplements if prescribed ( ) / *Ambil/berikan suplemen zat besi jika disuruh/perlu ( )* d. Treat other causes of anaemia (diseases and infections) – seek health-care assistance ( ) / *Rawat penyebab anemia lain (penyakit dan jangkitan) - dapatkan bantuan rawatan kesihatan ( )* e. Continue breastfeeding (for infants 6–23 months old) ( ) / *Teruskan penyusuan (untuk bayi 6-23 bulan) ( )* f. Other ( ) / *Lain-lain ( )* g. Don't know ( ) / *Tidak tahu ( )*
6. Can you list examples of foods rich in iron? *Bolehkah anda menyenaraikan contoh makanan yang kaya dengan zat besi?*	**a. Organ meat / *Daging organ*** i. Liver ( ) / *Hati ( )* ii. Heart ( ) / *Jantung ( )*

	b. Flesh meat / *Daging* i. Beef ( ) / *Lembu ( )* ii. Pork ( ) / *Babi ( )* iii. Lamb ( ) / *Kambing biri-biri ( )* iv. Goat ( ) / *Kambing ( )* v. Chicken ( ) / *Ayam ( )* vi. Duck ( ) / *Itik ( )* **c. Fish and seafood / *Ikan dan makanan laut*** i. Fresh fish ( ) / *Ikan segar ( )* ii. Dried fish ( ) / *Ikan kering ( )* iii. Canned fish ( ) / *Ikan dalam tin ( )* iv. Prawns ( ) / *Udang ( )* v. Shrimps ( ) / *Udang halus ( )* vi. Seafood ( ) *Makanan laut ( )* **d. Other ( ) / *Lain-lain ( )*** **e. Don't know ( ) / *Tidak tahu ( )***
7. When taken during meals, certain foods help the body absorb and use iron. What are those foods? *Apabila diambil semasa makan, makanan tertentu membantu tubuh badan menyerap dan menggunakan zat besi. Apakah makanan itu?*	a. Vitamin-C-rich foods, such as fresh citrus fruits (orange, lemons, lime, grapefruit, etc.) ( ) / *Makanan kaya dengan vitamin C, seperti buah sitrus segar (oren, lemon, limau, limau gedang, dll.) ( )* b. Other ( ) / *Lain-lain ( )* c. Don't know ( ) / *Tidak tahu ( )*
8. Some beverages decrease iron absorption when taken with meals. Which ones? *Sebilangan minuman mengurangkan penyerapan zat besi ketika diambil bersama makanan. Yang manakah satu?*	a. Coffee ( ) / *Kopi ( )* b. Tea ( ) / *Teh ( )* c. Other ( ) / *Lain-lain ( )* d. Don't know ( ) / *Tidak tahu ( )*

G(II): Attitude level / *G(II): Tahap sikap*

Instructions: Below is a list of statements dealing with your general attitude about IDA. Please indicate the correct answer for each statement. / *Arahan: Berikut adalah senarai pernyataan yang berkaitan dengan sikap umum anda mengenai anemia kekurangan zat besi. Sila nyatakan jawapan yang betul untuk setiap pernyataan.*

Statement ***Pernyataan***	**Answers** ***Jawapan***
1. How likely do you think you are to be iron-deficient/anaemic? *Sejauh manakah anda fikir anda mempunyai kekurangan zat besi / kekurangan kepekatan darah?*	a) Not likely ( ) / *Tidak mungkin ( )* b) You're not sure ( ) / *Anda tidak pasti ( )* c) Likely ( ) / *Mungkin ( )*
2. How serious do you think iron-deficiency/anaemia is? *Sejauh manakah anda menganggap kekurangan zat besi / anemia adalah serius?*	a) Not serious ( ) / *Tidak serius ( )* b) You're not sure ( ) / *Anda tidak pasti ( )* c) Serious ( ) / *Serius ( )*
3. How good do you think it is to prepare meals with iron-rich foods such as beef, chicken or liver? *Seberapa baik anda fikir menyediakan makanan dengan makanan kaya zat besi seperti daging lembu, ayam atau hati?*	a) Not good ( ) / *Tidak baik ( )* b) You're not sure ( ) / *Anda tidak pasti ( )* c) Good ( ) / *Baik ( )*
4. How difficult is it for you to prepare meals with iron-rich foods? *Betapa sukarnya untuk anda bagi menyediakan makanan dengan makanan kaya zat besi?*	a) Difficult ( ) / *Sukar ( )* b) So-so ( ) / *Sedang-sedang ( )* c) Not-difficult ( ) / *Tidak sukar ( )*
5. How confident do you feel in preparing meals with iron-rich foods? *Sejauh mana keyakinan anda dalam menyediakan makanan dengan makanan kaya zat besi?*	a) Not-confident ( ) / *Tidak yakin ( )* b) OK/So-so ( ) / *Ok/Sedang-sedang ( )* c) Confident ( ) / *Yakin ( )*
6. How much does you like the taste of iron-rich food item or meal?	a) Dislike ( ) / *Tidak suka ( )* b) You're not sure ( ) / *Anda tidak pasti ( )* c) Like ( ) / *Suka ( )*

Sejauh manakah anda suka rasa makanan yang kaya dengan zat besi?	

G(III): Practice level / *G(III): Tahap amalan*

Instructions: Below is a list of statements dealing with your general practice about IDA. Please indicate the correct answer for each statement. / *Arahan: Berikut adalah senarai pernyataan yang berkaitan dengan amalan umum anda mengenai anemia kekurangan zat besi. Sila nyatakan jawapan yang betul untuk setiap pernyataan.*

Statement ***Pernyataan***	**Answers** ***Jawapan***
1. Yesterday, during the day and night, did you eat any of the following? *Semalam, pada waktu siang dan malam, adakah anda makan makanan berikut?*	**a) Organ meat / *Daging organ*** i. Liver ( ) / *Hati ( )* ii. Heart ( ) / *Jantung ( )* **b) Flesh meat / *Daging*** i. Beef ( ) / *Lembu ( )* ii. Pork ( ) / *Babi ( )* iii. Lamb ( ) / *Kambing biri-biri ( )* iv. Goat ( ) / *Kambing ( )* v. Chicken ( ) / *Ayam ( )* vi. Duck ( ) / *Itik ( )* **c) Fish and seafood / *Ikan dan makanan laut*** i. Fresh fish ( ) / *Ikan segar ( )* ii. Dried fish ( ) / *Ikan kering ( )* iii. Canned fish ( ) / *Ikan dalam tin ( )* iv. Prawns ( ) / *Udang ( )* v. Shrimps ( ) / *Udang halus ( )* vi. Seafood ( ) *Makanan laut ( )* **d) Other** ( ) / ***Lain-lain ( )*** **e) No ( ) / *Tidak ( )***

2. Do you usually eat fresh citrus fruits, such as (orange, lemons, lime, grapefruit, etc) or drink juice made from them? *Adakah anda biasanya makan buah sitrus segar, seperti (oren, lemon, limau, limau gedang, dll) atau minum jus yang diperbuat daripada mereka?*	a) Yes ( ) / *Ya ( )* b) No ( ) / *Tidak ( )* c) Don't know / no answer ( ) / *Tidak tahu / tiada jawapan ( )*
If yes in Question 2, i. Consumption of vitamin C rich fruits every day? ***Sekiranya ya dalam Soalan 2,*** *i. Pengambilan buah-buahan kaya vitamin C setiap hari?*	a) Yes ( ) / *Ya ( )* b) No ( ) / *Tidak ( )* c) Don't know / no answer ( ) / *Tidak tahu / tiada jawapan ( )*
ii. When do you usually eat fresh citrus fruits? *ii. Bilakah anda biasanya makan buah sitrus segar?*	a) Before meal ( ) / *Sebelum makan ( )* b) During meal ( ) / *Semasa makan ( )* c) After meal ( ) / Selepas makan ( ) d) Other ( ) / *Lain-lain ( )* e) Don't know / no answer ( ) / *Tidak tahu / tiada jawapan ( )*
3. Consumption of tea/coffee usually? *Kebiasaan pengambilan teh/kopi?*	a) Yes ( ) / *Ya ( )* b) No ( ) / *Tidak ( )* c) Don't know ( ) / *Tidak tahu ( )*
If yes in Question 3, i. Consumption of tea/coffee every day? ***Sekiranya ya dalam Soalan 3,*** *i. Pengambilan teh / kopi setiap hari?*	a) Yes ( ) / *Ya ( )* b) No ( ) / *Tidak ( )* c) Don't know ( ) / *Tidak tahu ( )*
ii. When do you usually drink tea/coffee? *ii. Bilakah anda biasanya minum teh / kopi?*	a) 2 hours or more before a meal ( ) / *2 jam atau lebih sebelum makan ( )* b) Right before a meal ( ) / *Tepat sebelum makan ( )* c) During the meal ( ) / *Semasa makan ( )*

	d) Right after a meal ( ) / *Tepat selepas makan ( )* e) 2 hours or more after a meal ( ) / *2 jam atau lebih selepas makan ( )* f) Other ( ) / *Lain-lain ( )* g) Don’t know / no answer ( ) / *Tidak tahu / tiada jawapan ( )*

END OF QUESTIONNAIRE

SOALAN TAMAT

Appendix G: Turnitin report analysis

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