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Development and Validation of a Food Frequency Questionnaire for Vitamin D intake among Urban Pregnant Women in Malaysia

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ABSTRACT

Introduction: A culturally valid tool is lacking in order to assess vitamin D intake among Malaysians. This study aimed to develop and determine the validity and reliability of a semi-quantitative food frequency questionnaire (FFQ) for assessment of vitamin D intake among pregnant women living in urban areas of Malaysia. **Methods:** A total of 79 volunteer mothers (aged 18 to 40 years) in their first trimester of pregnancy participated in this study voluntarily. They completed both the 3-day 24-hour dietary recalls (3DR) and semi-quantitative food frequency questionnaires (FFQ1). The FFQ1 was validated against vitamin D intake calculated from the 3DR. After one week, 41 (51.89%) subjects completed the same FFQ1 (now called FFQ2) for reliability assessment. **Results:** The median vitamin D intake from 3DR was 117.6 IU/day with an interquartile range (IQR) of 54.8 to 193.7 IU/day. The median and IQR values from FFQ1 were 147.7 and 103.7 to 233.9 IU/day, respectively. The Spearman correlation coefficient was strong and positive, r=0.810 (p=0.01); cross-classification analyses revealed no misclassification and the Bland-Altman plot showed good agreement between the two dietary methods. The intra-class correlation (ICC) for reliability of vitamin D intake was 0.98; the cross-classification analysis showed 95.12% and was classified into the same quartile which supported a consistent habitual eating pattern. **Conclusion:** The new FFQ developed to assess vitamin D intake showed high correlation and high agreement with the majority of the sample population classified into the same quartile. It is concluded that the new FFQ is a valid and reliable screening tool for vitamin D intake for pregnant women in the urban areas of Malaysia.

Key words: FFQ, Malaysia, pregnant women, reliability, validation, Vitamin D

INTRODUCTION

A high prevalence of vitamin D deficiency is not expected in Malaysia, a tropical country with sunshine throughout the year. However, Moy & Bulgiba (2011) found that 87% of female adults in Malaysia had less than 50nmol/L of blood concentrations of vitamin D. Green *et al.* (2008) found that over 60% of non-pregnant women aged 18 to 40 years in Kuala Lumpur had

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25-hydroxy vitamin D (25(OH)D) levels below 50nmol/L. As part of a study of primary school children in Kuala Lumpur, Khor *et al.* (2011) found that 70.4% had low vitamin D with serum 25(OH)D levels of less than 50nmol/L.

Pregnant women are nutritionally vulnerable and any adverse circumstances during pregnancy will directly or indirectly affect the foetus. There is significant evidence that low maternal levels of 25(OH) D (vitamin D) are associated with adverse health outcomes for mothers, foetuses, neonates and children (Dror, 2011). Vitamin D deficiency during pregnancy has been associated with maternal problems such as gestational diabetes mellitus (GDM) (Alzaim & Wood, 2013), preeclampsia (Bodnar *et al.*, 2007), and an increased rate of caesarean section (Barrett & McElduff, 2010). Sufficient dietary vitamin D during pregnancy is important for foetal skeletal development (Leffelaar, Vrijkotte & van Eijsden, 2010; Mahon *et al.*, 2010; Robinson *et al.*, 2011) and tooth enamel formation (Purvis *et al.*, 1973). Lack of vitamin D during pregnancy can bring about long-term effects on infants such as congenital rickets (Innes *et al.*, 2002).

Humans obtain vitamin D from three sources: sunlight, dietary supplements, and food. Most Malaysians meet their vitamin D needs through exposure to sunlight as few foods available in Malaysia contain vitamin D. Most dietary vitamin D comes from fortified food such as milk, margarine and cereals. In health care practice, pregnant women are encouraged to increase their calcium intake. However, without adequate intake of vitamin D, calcium absorption is low. The recommended vitamin D intake for pregnant women in Malaysia is 200 IU/ day, which is lower than that of other countries. The minimum recommended intake has been as high as 600 IU/day of vitamin D, and at least 1500-2000 IU/day may be needed to maintain a blood level of 25(OH)D above 75nmol/L (Holick *et al.*, 2011).

There have been several food frequency questionnaire (FFQ) validation studies related to vitamin D intake worldwide (Park *et al.*, 2013; Pritchard *et al.*, 2010; Taylor *et al.*, 2009). Nevertheless, different countries have their own type of foods rich in vitamin D. A newly developed FFQ requires a lot of references, surveys and some of the foods would require laboratory testing for vitamin D content. Therefore, as there is an unexpectedly high prevalence of vitamin D deficiency among Malaysians, a valid screening tool is essential to assess the population's vitamin D intake, especially for vulnerable groups such as pregnant women. This study aimed to develop and validate a semi-quantitative FFQ against a 3-day 24-hour dietary recall (3DR).

METHODS

Development of a new semi quantitative FFQ for vitamin D intake

The new FFQ was developed based on the FFQ in the Malaysian Adult Nutrition Survey (MAN) (Safiah *et al.*, 2003) and the FFQ for pregnant women in Malaysia (Loy *et al.*, 2011). A list of foods containing vitamin D was prepared based on: (i) the Nutritionist Pro software (Smolin & Grosvenor, 2008); (ii) selected-food composition tables (Health Canada, 2012; Holick, 2007; Pearce & Cheetham, 2010; Taylor *et al.*, 2009; USDA, 2012); and (iii) food label (Legal Research Board 2014). Informal surveys were carried out at two hypermarkets to confirm the labels on foods containing vitamin D. Malaysian foods that were fortified with vitamin D were listed on the guideline for calculating vitamin D intake in the new FFQ and 3DR. Food items that are commonly consumed in Malaysia but are neither listed in any references mentioned above nor have their vitamin D content clearly declared were sent for vitamin D content determination at private laboratories approved by the Ministry of Health Malaysia.

A list was prepared containing natural food and vitamin D fortified foods. A third category included in this list was vitamin D dietary supplements. In order to improve the questionnaire in terms of wording and spelling, face validity was done among a sample of ten women. On average, a total 15 to 20 minutes was spent by these subjects to complete the questionnaire. This was followed by content validity evaluation by three nutrition experts to ensure that the questionnaire covered all foods containing vitamin D consumed in Malaysia. Subsequently, back-to-back translation was done by two translators who were good in both languages, Malay and English. Lastly, some corrections were made and the final questionnaire was developed.

Subjects and sampling

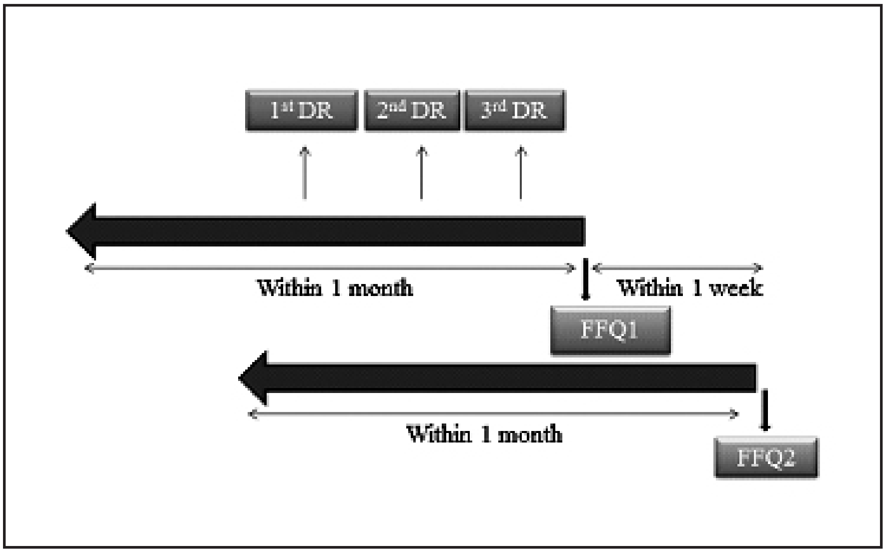
This study was conducted in an urban district in Selangor, Malaysia. There are six clinics in the district. Three clinics were selected for the main study and one clinic was selected for the pilot study. A total of 79 pregnant women voluntarily enrolled in this study (from September to mid-October 2013) and completed both the FFQ1 and 3DR. The inclusion criteria in this study were 18 to 40-year-old Malaysian mothers who were in the first trimester of gestation based on their last menstrual periods or from their ultrasound scans. Pregnant women who were fasting or on diet restrictions were not included in this study as they would have influenced the results of the validation. The exclusion criteria for mothers included having type I or type II diabetes, hypertension, parathyroid disease or uncontrolled thyroid disease, rheumatoid arthritis, metabolic bone disease, mal-absorption, kidney disease, liver disease, treatment with antiepileptic drugs, and tuberculosis. Both the pilot and main study had the same inclusion and exclusion criteria that would not influence the serum vitamin D level. Written informed consent was obtained from all subjects.

The subjects were asked to recall the foods they consumed noting the ingredients, portion size and the cooking method. Only subjects who were interested and willing to participate in this study were recruited since self-selected study participants have been found to give more accurate and frank answers (Riboli *et al*., 1997). The current intake of dietary supplements was included in FFQ1 and subjects were asked to notify if they took any dietary supplements in the 3DR period. They were asked to complete the interviewer-administered FFQ1 and 3DR (two weekdays and one weekend day). However, only 41 out of 79 (51.8%) subjects agreed to repeat the FFQ1 after one week, as many subjects were working and were not able to attend the reliability test.

Data Collection

3-day 24-hour dietary recall (3DR)

The 3DR was chosen as it was more convenient for the subjects. A five-day dietary recall could be too tedious and less accurate due to the longer duration involved. In the 3DR, subjects were asked about the food they ate, how the food was prepared (e.g., frying, boiling, grilling, steaming, etc.), portion size, and the ingredients used to help give a more accurate estimation of vitamin D intake. The FFQ1 was given after completing the 3DR to ensure that the 3DR assessment was within one month of the FFQ1 (Figure 1). The timing between the two data gathering phases aimed to minimise the impact of changes to eating habits due to factors such as eating special foods during festive seasons. All subjects were given an explanation at the start of this study relating to the FFQ1 and 3DR by a trained interviewer.



1st DR, 2nd DR, 3rd DR: First day, second day and third day dietary recall

Figure 1. The administration structure of the new FFQ (FFQ1) and the 3DR - FFQ1 was given after the 3DR was completed to ensure that the 3DR assessment was within one month of the first FFQ1 assessment. The FFQ1 (FFQ2) was then repeated within one week.

New-semi quantitative food frequency questionnaire (FFQ)

The new FFQ focused on the habitual eating pattern of the subjects and its impact on vitamin D intake. The foods were classified into 3 categories: (i) foods that were fortified with vitamin D; (ii) foods that naturally contained vitamin D; and (iii) dietary supplements. The new FFQ contained forty-five food items with vitamin D content of 10 IU or more and thirteen types of supplements. The criteria for selecting the minimum level of vitamin D content in the listed foods were similar to the study done by Pritchard *et al.* (2010). The new FFQ required subjects to recall and record the frequency of food intake within a day, a week or a month period. The portion size was also recorded with reference to the size of the dish. A medium-sized dish was shown to help the respondent determine the size of the portion of food or drink consumed. Portions, half the size of the medium-sized dish, were considered small. Portions, 50% larger than the medium-sized dish, were considered to be large. During the session, a photograph album (Institut Kesihatan Umum, 2011; Suzana *et al.*, 2009) was used as a tool to assist the subjects in identifying the serving sizes of the food.

Statistical analysis

All data were analysed using the IBM SPSS Version 22.0. All results were reported using 95% confidence intervals and significance levels with *p*-values < 0.05. Only completed questionnaires were analysed. The amount of vitamin D consumed by subjects from food sources in a day was calculated. Vitamin D intake or nutrient values for each food item were calculated using Nutritionist Pro™ Diet Analysis Software. Some of the nutrient values were taken from the National Nutrient Database for Standard Reference, United States Department of Agriculture (USDA), and the Canada Nutrient File

(CNF) (Health Canada 2012; USDA 2012). Meanwhile, the fortified food and dietary supplements with vitamin D content were calculated manually based on product label information using Microsoft Office Excel 2007. Microsoft Office Excel 2007 was also used to calculate the weight (in grams) of each food item depending on the frequency of intake. Some of the Malaysian foods that were sent for vitamin D analysis were calculated manually based on the vitamin D content per 100g.

Validity

Vitamin D levels were not normally distributed for both FFQ and average 3DR. The Wilcoxon Signed Rank Test was used to analyse the differences between the two methods. The Spearman Correlation Coefficient was used to evaluate the strength and relationship between the two dietary methods. The cross-classification analysis was used to evaluate the ability of both methods to be classified into the same quartile, ± one quartile or misclassification. Each of the dietary methods was classified into four quartiles (5th, median [50th] and 95th percentiles). The Bland-Altman analysis was used to assess the agreement between two quantitative measurements (FFQ and 3DR) since a high correlation does not imply agreement. This analysis also calculated the mean difference between two measurement methods. The difference of the paired measurements was plotted against the mean of the two measurements. It is recommended that 95% of data points should lie within the ±2 standard deviations (SD) of the mean difference (Lei, Zaloudick & Vorechovsky, 2002). The cut-off point of vitamin D intake followed the Recommended Nutrient Intakes (RNI) for Malaysia. The RNI for vitamin D intake during pregnancy in Malaysia is 200 IU/day or 5 μg/day (MOH, 2005). The sensitivity of this study was defined as the proportion of subjects detected with vitamin D intakes below the RNI based on the FFQ1 and the 3DR. Specificity was defined as the proportion of subjects with vitamin D intakes ≥200 IU/day.

Reliability

The Wilcoxon Signed Rank Test was used to test the difference between the FFQ1 and the FFQ2. Test-retest (intra–class correlation) was done between the FFQ1 and the FFQ2 assuming there were no significant changes in the results between the two occasions. Cross-classification analysis was done to evaluate misclassification of vitamin D intake. Both vitamin D intakes from FFQ1 and FFQ2 were computed into four quartiles (5th, median [50th] and 95th percentiles).

Ethical approval

This study was approved by the Medical Research and Ethics Committee of the National University of Malaysia (FF-039-2013) and the Medical Research and Ethics Committee (MREC) of the Ministry of Health (NMRR-12-1156-14424).

RESULTS AND DISCUSSION

The FFQ for vitamin D intake covered the foods that were high in vitamin D content but did not assess the intake of other nutrients. The FFQ was developed from the Malaysian Adult Nutrition Survey FFQ and FFQ for pregnant women in Malaysia that included a number of food items that had specific nutrients with high vitamin D content. The cultural and religious aspects of the population studied must be considered when developing a FFQ with regard to their choice of fortified foods, and food consumed during festive periods pertaining to their culture or religion.

Surveys in local hypermarkets

Informal surveys and observations at two local hypermarkets showed that most of the milk brands sold were fortified with vitamin D. According to the Malaysian

Food Act 1983, each 100 grams of margarine shall contain no less than 250 IU and not more than 350 IU vitamin D. The maximum recommended amount of daily serving for food fortified with vitamin D in Malaysia is 800 IU (Legal Research Board 2014). Foods claiming to be enriched, fortified, supplemented or strengthened with specific vitamins (e.g. vitamin D) required mandatory nutrition labelling under Regulation 26(7). Additionally, the vitamin or mineral content must be at least 5% of the Nutrient Reference Value (NRV) per serving. For example, if NRV for vitamin D is 200 IU, it required that the fortified food has at least 10 IU of vitamin D per serving (MOH, 2010). Several brands of butter, yoghurt, cereal, soy milk and bread are fortified with vitamin D in Malaysia. Fresh soy bought from hawkers did not contain vitamin D. Flour, cooking oil, macaroni and rice are not fortified with vitamin D in Malaysia. These findings were similar to those found by Calvo, Whiting & Barton (2004) who claimed that macaroni, rice and cornmeal products are not fortified with vitamin D in the USA.

In the USA and Canada, fortified food and dietary supplements contribute most of the populations' vitamin D requirements. This is due to the limited number of foods naturally rich in vitamin D and limited sunlight exposure people have during the day, especially in winter. In the USA, the main sources of vitamin D are milk and breakfast cereals, whereas in Canada, the sources are fortified milk and margarine (Calvo *et al.*, 2004). The situation in Malaysia is similar to Canada, where it is mandatory for margarine to be fortified with vitamin D, and milk products are the predominant sources for vitamin D. This study observed that cheese, ice-cream, mayonnaise and baking products (e.g., whipped cream) claimed to not contain vitamin D. It is possible that these products have vitamin D contents of less than 10 IU. This is similar to Canada, where industrial milk, normally used in baking and other milk products such as cheeses, does not have to be fortified, while some food products such as yoghurt may use fortified milk during their manufacture (Calvo *et al.*, 2004). It is highlighted that not all dairy products are enriched with vitamin D (Calvo, 2000). Fruit juice drinks in Malaysia are not fortified with vitamin D, but claim in their labels to have other vitamins such as A, C, and/or E. The situation is different in Canada, where some fruit juice drinks are fortified with vitamin D (Calvo *et al.*, 2004). Taylor *et al*. (2009) also found no vitamin D in calcium fortified juices (orange and others). Puddings, pancakes, waffles and homemade ice cream are high in vitamin D because their main ingredients are fortified milk and eggs.

Food sampling for vitamin D analysis

Foods which are naturally rich in vitamin D are limited in Malaysia. Natural foods rich in vitamin D are fatty fish, fish oil, eggs, shrimp, meat pork, chicken liver, beef liver, mushrooms, and egg yolks. The types of fish that are rich in vitamin D are salmon, tuna, sardines, herring, and mackerel (Health Canada, 2012). In Malaysia, tuna is also known as *ikan tongkol aya/kayu* but the size is smaller compared to the tuna species from other parts of the world. Two types of mackerels common in Malaysia include the Spanish mackerel *(ikan tenggiri*) and Indian mackerel (*ikan kembung/ mabong/pelaling or temenung*) (Atan, Jaafar & Abdul Rahman, 2010; Tee *et al.*, 1997). Sampled foods were analysed as some of the food items that are commonly eaten by Malaysians do not document vitamin D content data or there is a lack of clarity in the published references. Table 1 shows some of the food samples that were sent to a private laboratory to have their vitamin D content determined.

Pilot study

Out of the ninety-five pregnant women recruited, seventy-nine (83.2%) successfully completed both the 3DR and FFQ1. Sixteen

Table 1. Vitamin D content of some food samples analysed in a private laboratory.

Food items (100g)	*Vitamin D2 +D3* μg*/100g*	*IU/100g*
Ikan kembung/Indian Mackerel	0.7	28.0
Ikan tongkol	35.8	1432.0
Chicken meat with skin	2.1	84.0
Prawn	4.1	164.0
Belacan (shrimps or small fish that have been salted and dried in the sun for flavouring food)	25.1	1004.0
Anchovies	20.6	824.0

subjects failed to complete both the 3DR and FFQ. Based on other studies, Pritchard *et al.* (2010) found that a suitable sample size for FFQ validation studies is about fifty to a hundred subjects.

The mean (±SD) age of the subjects was 28.61 (±4.7) years. About 89.9% were Malays, 2.5% Chinese, 3.8% Indian and 3.8% were of other ethnic backgrounds. Only 31 (39%) of the seventy-nine subjects took vitamin supplements (e.g., vitamin D, New Obimin®, Pramilet®, Omega Plus®, Shaklee®, Elken® spirulina, fish oil, Neurogain®, etc.). Most of them had just started on prenatal vitamin supplements. Only two took vitamin D supplements for more than a month. All vitamin D supplements were included in the calculation for dietary vitamin D intake. Fifty-three (67.1%) pregnant women failed to meet the Malaysian RNI for vitamin D (200 IU/day), whilst only twenty-six (32.9%) pregnant women had vitamin D intake ≥200 IU/day based on the new FFQ.

Validity

In this study, vitamin D intake was assessed using the FFQ1 and 3DR. The results showed that the median and interquartile range (IQR 25th, 75th percentile) for vitamin D intake for 3DR and FFQ1 were 117.6 (54.8,193.7) IU/day and 147.7 (103.7, 233.9) IU/day, respectively. The Wilcoxon Signed Rank Test showed that the FFQ1 over-estimated vitamin D intake when compared to 3DR (Z=-5.273, p=0.001). It was found that vitamin D intake based on the two dietary methods were significantly different, where the FFQ1 tended to over-estimate the vitamin D intake. However, using the Spearman correlation coefficient, the intake derived from the FFQ1 was significantly correlated with intake based on 3DR (r= 0.810, p=0.01). Thus, there was a strong and positive correlation between the two dietary methods. A study done by Pritchard *et al*. (2010) among post-menopausal women in Canada claimed that Pearson correlation coefficient for vitamin D intake was 0.89, similar to the findings of this study. Papandreou *et al.* (2014) reported that FFQ validation for vitamin D and calcium among thirty-five female students in Abu Dhabi also showed a strong, positive relationship between the FFQ and 3DR for vitamin D intake (r = 0.82, p <0.001). Furthermore, their study showed that there was no difference in mean intake of vitamin D regardless of the reporting methods used. This indicates that the subjects consistently reported the food items that they consumed. However, this study showed that the FFQ1 over-estimated vitamin D intake compared to 3DR.

Cross-classification analyses showed that 78.5% of vitamin D intake was classified into the same quartile, 21.5% was

Table 2. Cross-classification analyses between dietary vitamin D intake based on the FFQ1 and the 3DR (n=79).

	% classified into same quartile	*% classified into same ± 1 quartile*	*% misclassified*
Vitamin D intake	62 (78.5%)	17 (21.5%)	0

Quartiles 1 to 4 for FFQ1 were <40.239, 40.239-147.670, 147.671-369.354, >369.354
Quartiles 1 to 4 for 3DR were <14.209, 14.209-117.646, 147.647-352.937, >352.937

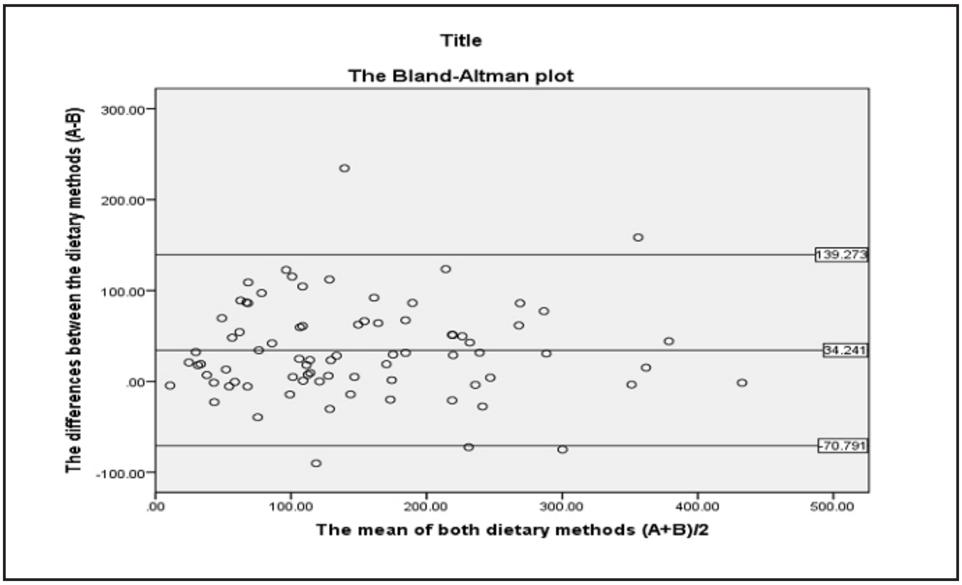


Figure 2. Bland Altman analysis comparing the FFQ1 with the 3DR for vitamin D intake

classified into the adjacent (+1/-1) quartiles and none were misclassified (Table 2). This showed that, compared to the 3DR, the FFQ1 distributed the vitamin D dietary intake correctly into the same and adjacent quartiles.

The Bland-Altman method was used to evaluate the agreement between two different dietary intake instruments and to measure the mean difference between the FFQ1 and 3DR. The Bland-Altman plot showed that the mean (±SD) difference between these two dietary methods was 34.241 (±52.516). This resulted in the ± 2SD of the means being 70.791 and 139.273. About 95% of the points were within ± 2SD with 4/79 outliers (Figure 2). The mean difference of vitamin D intake in this study, 34.241 IU/day, was smaller compared to the study done by Pritchard *et al.*(2010) among Canadian post-menopausal women in which the mean difference was 75 IU/day. The high mean difference in the Canadian post-menopausal women could be due to a small sample size (n=15), the age, and the health status of the population. The low mean difference in this study may be due to not knowing the study objectives.

Table 3. Cross-classification analyses in dietary vitamin D intake between FFQ1 and FFQ2. (n=41).

	n (%) classified into the same quartile	*n (%) classified into the adjacent ± 1 quartile*	*n (%) misclassified*
Vitamin D intake	39 (95.1%)	2 (4.9%)	0

Quartiles 1 to 4 for FFQ1 were <35.566, 35.566-136.702, 136.703-363.607, >363.607
Quartiles 1 to 4 for FFQ2 were <46.176, 46.176-140.444, 140.445-372.986, >372.986

Subjects who were on dietary restrictions or fasting during the 3DR were excluded and emphasis was given to the brand name of foodstuffs in order to detect vitamin D intake.

There is no gold standard directly to assess the validity of FFQ, but the commonly used parameters are weight record or 24-hour dietary recall (Cade *et al.*, 2002). In this study, the FFQ1 to assess vitamin D intake was compared against the 3DR. The FFQ1 was able to detect 52 out of 60 subjects who had vitamin D intake of less than 200 IU/day based on the 3DR. Meanwhile, the FFQ1 has the ability to identify eighteen out of nineteen subjects who had vitamin D intake ≥ 200 IU/day. This gave a sensitivity of 86.7% and a specificity of 94.7%. The positive and negative predicted values of the tests were 98.1% and 69.2%, respectively.

Reliability

For test-retest reliability, 41 of the 79 subjects (51.89%), repeated and completed their FFQ (FFQ2). The Wilcoxon Signed Rank Test showed that there was no significant difference in vitamin D intake between FFQ1 and FFQ2 (Z=-1.782, p=0.075). The intra-class correlation (ICC) for vitamin D intake between the two FFQs was 0.98. The cross-classification analyses showed that 95.1% of vitamin D levels were classified into the same quartile, 4.9% into the adjacent (+1/-1) quartiles, and there was no misclassification (Table 3). This study found that in the repeated FFQ1 and FFQ2, the highest percentage of subjects fell into the same quartile (95.1%) compared to FFQ1 against 3DR, which was 78.5%. The higher percentage in the repeated FFQ1 and FFQ2 may be due to the subjects really knowing what they eat and what they do not eat because it focused on habitual intake of vitamin D. Both FFQ and 3DR had their own recall bias that is difficult to exclude from this study.

The strengths of this study are: (1) this was the first validated semi-quantitative FFQ for vitamin D intake in Malaysia; (2) the vitamin D content was measured in some of the food items commonly consumed by the Malaysian population; (3) an unofficial survey was done in two local hypermarkets to clarify vitamin D fortified foods and to provide a list of vitamin D fortified foods with their brand names and nutrient content; (4) data collection was carried out by a well-trained interviewer who administered the FFQ and the 3DR using a food album; and (5) a list of supplements was added to the FFQ. The new FFQ was easier, simpler and more convenient to use because it was based on eating habits. This was in contrast to a 24-hour dietary recall, where the subjects have to recall every food item that they have consumed.

CONCLUSION

The data derived from the new FFQ concurs well with the data from 3DR with good validity and reliability. The results suggest that the new FFQ can be effectively used to assess daily vitamin D intake not only for pregnant women but also for other populations. The new FFQ is a valid and reliable tool that can be used as a screening tool in epidemiologic studies as well as in clinical settings.

Conflict of interest

The authors declare that they have no competing interests.

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