VITAMIN D LEVEL AND ITS ASSOCIATION WITH ADIPOSITY AMONG MULTI ETHNIC TEACHERS IN WILAYAH PERSEKUTUAN KUALA LUMPUR, MALAYSIA

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FACULTY OF MEDICINE UNIVERSITY OF MALAYA KUALA LUMPUR

2016

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICAL SCIENCE

FACULTY OF MEDICINE UNIVERSITY OF MALAYA KUALA LUMPUR

2016

UNIVERSITY OF MALAYA

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Name of Degree: Master of Medical Science

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

Vitamin D level and its association with Adiposity among Multi Ethnic

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ABSTRACT

Vitamin D plays an essential role in health. Its deficiency can not only increase the risk of osteoporosis, but also contribute to cardiovascular diseases, diabetes and certain types of cancers. Skin synthesis of vitamin D from sunlight exposure constitutes the major source of vitamin D. Evidences showed that not only temperate countries experience the problem of vitamin D deficiency, but a similar problem exists in tropical countries like Malaysia, Indonesia, Saudi Arabia and Tehran. Currently, there are new evidences that show obesity may contribute to vitamin D deficiency. Obesity-associated vitamin D deficiency is most likely due to the decreased bioavailability of vitamin D_3 because of its deposition in the body fat compartments.

The aim of this study was to identify the risk factors contributing to a low serum 25(OH)D level and its association with adiposity among secondary school teachers in the state of Wilayah Persekutuan Kuala Lumpur (WPKL). This was a cross sectional study of two-stage sampling conducted from February 2013 to May 2013. First, 50% out of a total of 80 government day schools in WPKL were randomly selected from each district in Pudu, Bangsar, Sentul and Keramat respectively. Then, all teachers from the selected schools who fulfilled the inclusion criteria of the study were invited to participate. Ethics clearance was obtained from the University Malaya Medical Centre (UMMC) Ethics Committee (Reference Number: 950.1). Approval from the Ministry of Education, Education Board of Wilayah Persekutuan Kuala Lumpur (WPKL) and principals from each selected schools were obtained before data collection. Informed consent was obtained from all participants. The data collection included serum 25-hydroxyvitamin D (25(OH)D), Parathyroid Hormone (PTH), blood glucose, fat percentage, waist circumference and Body Mass Index (BMI). Demographic

characteristics, sun exposure and avoidance, and level of physical activity were also collected using a self-administered questionnaire.

A total of 858 participants were recruited. The majority of them were Malays, females and married. The overall prevalence of vitamin D deficiency (<20 ng/ml) was 67.4%. Indian (80.9%) participants had the highest proportion of vitamin D deficiency, followed by Malays (75.6%), others (44.9%) and Chinese (25.1%). There was a significant negative association between serum 25(OH)D level with BMI (β = -0.23) and body fat percentage (β = -0.14). In the multivariate linear regression analysis; Malays, Indians, females (p<0.001), higher BMI and larger waist circumference (p<0.05) were significantly associated with lower serum 25(OH)D level. The full model explained 32.8% of variation contributing to serum 25(OH)D level among the participants. Adiposity explained only less than 1% of the variability of serum 25(OH)D level. The two most influential factors affecting serum 25(OH)D level were ethnicity and sex. Health education should be targeted in weight management, sex based behaviors on sun exposure and avoidance, as skin pigmentation is non-modifiable.

ABSTRAK

Vitamin D memainkan peranan yang penting kepada kesihatan. Kekurangan vitamin D bukan sahaja boleh meningkatkan risiko osteoporosis, bahkan juga menyumbang kepada penyakit kardiovaskular, diabetes dan beberapa jenis kanser. Sintesis vitamin D oleh kulit daripada pendedahan cahaya matahari merupakan sumber utama vitamin D. Bukti-bukti menunjukkan bahawa bukan sahaja negara-negara yang beriklim sederhana mengalami masalah kekurangan vitamin D, masalah juga wujud di negara-negara tropika seperti Malaysia, Indonesia, Arab Saudi dan Tehran. Obesiti berkemungkinan menyumbang kepada kekurangan vitamin D yang disebabkan oleh pemendapan vitamin D dalam lemak di dalam badan.

Kajian ini dilakukan bertujuan untuk mengenal pasti faktor-faktor yang menyumbang kepada kekurangan serum 25(OH)D dan perkaitannya dengan obesiti dalam kalangan guru sekolah menengah di Wilayah Persekutuan Kuala Lumpur (WPKL). Kajian ini merupakan kajian keratan rentas pensampelan dua peringkat yang dilakukan dari bulan Februari 2013 hingga Mei 2013. Yang pertama, 50% daripada 80 buah sekolah kerajaan harian di WPKL telah dipilih secara rawak dari setiap daerah; Pudu, Bangsar, Sentul, dan Keramat. Kemudian, semua guru yang memenuhi syarat-syarat kajian dari sekolah-sekolah yang terpilih telah dijemput untuk mengambil bahagian dalam kajian ini. Kelulusan telah diperoleh daripada Jawatankuasa Etika Pusat Perubatan Universiti Malaya (PPUM) (Nombor Rujukan: 950.1). Kelulusan daripada pihak Kementerian Pendidikan Malaysia, Jabatan Pendidikan Negeri dan pengetua-pengetua dari setiap sekolah yang terpilih telah diperoleh sebelum kerja-kerja pengumpulan data dilakukan. Persetujuan penglibatan daripada setiap peserta juga turut diperoleh. Pengumpulan data termasuklah serum 25-hydroxyvitamin D (25(OH)D), hormon paratiroid (PTH), glukosa darah, peratusan lemak badan, lilitan pinggang dan Indeks Jisim Badan (BMI). Ciri-ciri

demografik, soal selidik pendedahan dan perlindungan cahaya matahari, dan tahap aktiviti fizikal juga turut dikumpulkan dengan menggunakan soal selidik yang telah disahkan.

Seramai 858 orang guru telah mengambil bahagian dalam kajian ini. Majoriti terdiri daripada etnik Melayu, perempuan dan sudah berkahwin. Peratusan keseluruhan kekurangan vitamin D (<20 ng/ml) adalah sebanyak 67.4%. Etnik India mencatatkan peratusan tertinggi (80.9%) kekurangan vitamin D yang diikuti oleh etnik Melayu (75.6%), lain-lain (44.9%) dan Cina (25.1%). Terdapat perhubungan yang negatif antara serum 25(OH)D dengan BMI (β = -0.23) dan peratusan lemak (β = -0.14). Dalam analisis multivariat regrasi linear; etnik Melayu, etnik India, perempuan (p<0.001), BMI yang lebih tinggi dan ukur lilit pinggang yang lebih besar (p<0.05) menunjukkan perhubungan yang signifikan dengan kekurangan serum 25(OH)D. Model penuh menjelaskan sebanyak 32.8% variasi yang menyumbang kepada serum 25(OH)D. Dua faktor yang paling mempengaruhi tahap serum 25(OH)D ialah etnik dan jantina. Pendidikan kesihatan perlu disasarkan dalam pengurusan berat badan dan tingkah laku berdasarkan jantina dalam pendedahan kepada cahaya matahari memandangkan faktor pigmentasi kulit tidak boleh diubah suai.

ACKNOWLEDGEMENTS

First of all, I would like to express my sincerest gratitude to my supervisor, Associate Professor Dr Moy Foong Ming from the Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya for giving me this opportunity to work on this project. Her knowledge, experience, guidance and support have encouraged me throughout my research till the very end. This thesis writing would not have been possible without her personal guidance. I am sure this thanks is not enough to express how much I appreciate her.

Besides that, I would like to express my warm and sincere thanks to Associate Professor Dr Nirmala Bhoo Pathy, Dr Marzuki Isahak, Dr Rafdzah and Dr Hazreen Bin Abdul Majid from Department of Social and Preventive Medicine and Dr Shirene Vethakka from Department of Medicine, University of Malaya for their valuable insight, support and guidance during my Master's presentation. Their criticisms and advice had a remarkable influence on my research.

I am tremendously thankful to my beloved parents, husband, daughter and all my family members for their endless support. They have always been my source of spirit and motivation along this study. Thanks as well to my colleagues and friends who were directly or indirectly involved in this study. Thank you for always enduring my stress and support me in all that I do.

Thank you God for always being with me along this journey.

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LIST OF SYMBOLS

< Less than

- > Greater than
- \leq Less than or equal to
- \geq Greater than or equal to
- °C Degrees celcius
- ± Plus- minus
- *r* Correlation coefficient
- μl Microlitre

LIST OF ABBREVIATIONS

BF	Body Fat
BIA	Bioelectrical Impedance Analyzer
BMI	Body Mass Index
CI	Confident Interval
CDL	Clinical Diagnostic Lab
CHD	Coronary Heart Disease
CLIA	Chemiluminescence
cm	Centimetre
CNS	Central Nervous System
CV	Coefficient of Variation
CVD	Cardiovascular Disease
DEQAS	Vitamin D External Quality Assessment Scheme
DXA	Dual-energy X-ray absorptiometry
FOM	Faculty of Medicine
HPLC	High Performance Liquid Chromatography
ICC	Intraclass Correlation
IOM	Institute of Medicine
IPAQ	International Physical Activity Questionnaire
IU	International Unit
IQR	Interquatile Range
kg	Kilogram
LC	Liquid Chromatography
MET	Metabolic equivalent
MI	Myocardial Infarction
MS	Mass Spectrometry
NHMS III	Nationwide Third National Health and Morbidity Survey
NIH	National Institute of Health
PA	Physical Activity
PTH	Parathyroid Hormone
RIA	Radioimmunoassay
RPM	Revolution per minute
SD	Standard deviation
SPSS	Statistical Packages for the Social Sciences

- UMMC University Malaya Medical Centre
- UVB Ultra-violet B
- WC Waist circumference
- WHR Waist-to-hip ratio
- WHO World Health Organization
- WHtR Waist-to-height ratio

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CHAPTER 1: INTRODUCTION

1.1 Definition of vitamin D

Vitamin D comprises of a group of fat soluble seco-sterols. It is available in two different forms, which are ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3), synthesized by plants and human respectively under the exposure to ultraviolet B rays of sunlight. Vitamin D is synthesized in the skin through sunlight exposure as this is the primary source of vitamin D for most people. It is also acquired from diet where it naturally occurs in fatty fish and eggs (Holick, 2007).

1.2 Benefits of vitamin D on health

Vitamin D plays an essential role to our health. The major biologic function of vitamin D is to maintain normal blood levels of calcium and phosphorus in the human body. It assists in calcium homeostasis and aids in bone mineralisation (Lips, Duong, Oleksik, Black, Cummings, Cox et al., 2001). In the recent years, the roles of vitamin D have expanded due to the increase of evidence in which adequate vitamin D has been associated with a decrease in the risk of getting non-skeletal chronic diseases such as cardiovascular disease, certain types of cancer, autoimmune disease, infectious diseases, hypertension, type 2-diabetes and some mental and neurological health conditions (Munger, Levin, Hollis, Howard, & Ascherio, 2006; Pittas, Dawson-Hughes, Li, Van Dam, Willett, Manson et al., 2006; Rosen, Adams, Bikle, Black, Demay, Manson et al., 2012). In the Nurses' Health Study conducted among 83,000 individuals, those who took at least 800 IU of vitamin D daily had 33% lower risk of developing type II diabetes (Pittas, Dawson-Hughes, Li, Van Dam, Willett, Manson et al., 2006). Prospective and retrospective epidemiological studies showed that individuals with a low serum 25(OH)D level had a higher risk of getting prostate and breast cancer (Holick, 2007).

1.3 Prevalence of vitamin D deficiency in Europe and Asia

Vitamin D is a major public health problem that affects almost one billion people worldwide. In 2010, low vitamin D intake in a large sample of healthy middle age men and women resulted in 29% having vitamin D deficiency (<20 ng/ml) (Brock, Huang, Fraser, Ke, Tseng, Stolzenberg-Solomon et al., 2010). In North India, 78% of healthy hospital staff had vitamin D deficiency (<20 ng/ml) (Arya, Bhambri, Godbole, & Mithal, 2004). A study among middle aged South Koreans reported that almost 64.5% of Korean females suffered from vitamin D deficiency (<30 ng/ml) (Kim, 2009). In 2011, a study carried out among the Thailand population showed that 64.4% of them had vitamin D deficiency (<30 ng/ml) (Chailurkit, Aekplakorn, & Ongphiphadhanakul, 2011).

Although there are not many studies carried out on vitamin D in Malaysia compared to European countries, there are a few evidences that showed that vitamin D deficiency among Malaysian should be taken into serious matter. The earliest study was carried out in 2004 among postmenopausal Malaysian women which showed that 71% of Malay women had vitamin D insufficiency (10-20 ng/ml) (Rahman, Chee, Yassin, & Chan, 2004). This was followed by the study done in 2008 among women of child-bearing age living in Jakarta and Kuala

Lumpur which reported that 60% of them suffered from vitamin D deficiency (<20 ng/ml) (Green, Skeaf, Rockell, B.J., Lambert, Todd et al., 2008). Later, a study in 2011 among Malay adults in Kuala Lumpur showed that 70% of them had vitamin D deficiency (<20 ng/ml) (Moy & Bulgiba, 2011). The study by Nurbazlin et al. (2013) reported that 48% of women in urban areas suffered from vitamin D deficiency (<12 ng/ml) while the study by Chin et al. (2014) which focused on Malay and Chinese men living in Klang valley showed that the prevalence of vitamin D insufficiency (12-20 ng/ml) and vitamin D deficiency (<12 ng/ml) were 22.7% and 0.5% respectively. Overall, vitamin D deficiency especially among Malaysian females is high and severe.

1.4 Population at risk of vitamin D deficiency

Vitamin D deficiency is common in temperate countries, especially during winter due to poor sunlight exposure (Gordon, Depeter, Feldman, Grace, & Emans, 2004; Rajakumar, De Las Heras, Chen, Lee, Holick, & Arslanian, 2011). A study among healthy western Canadians showed that there was a significant rise in serum 25(OH)D level during spring and summer, but it declined to a significantly lower level during the winter months (Rucker, 2001).

Women are the individuals at risk of having vitamin D deficiency, especially those with pregnancy, osteoporosis and menopause. In North India, approximately 84% of pregnant women had vitamin D deficiency (<20 ng/ml) (Arya et al, 2004). Worldwide, approximately 64% of menopausal women with osteoporosis had serum 25(OH)D levels <30 ng/ml (Lips, Hosking, Lippuner, Norquist, Wehren, Maalouf et al., 2006). Prevalence of hypovitaminosis D (<30 ng/ml) among postmenopausal women were 49% in Malaysia, 47% in Thailand, 92% in South Korea and 90% in Japan (Lim, Kung, Sompongse, Soontrapa, & Tsai, 2007).

Individuals with darker skin are susceptible to vitamin D deficiency. A darker skin colour might inhibit vitamin D from being synthesized in the body. The study conducted by Holick (2004) reported that 30% of free living white, 42% of Hispanic and 84% of black elderly had vitamin D deficiency (<20 ng/ml) among the United States (US) population.

Inactive individuals are also at higher risks of getting vitamin D deficiency due to less exposure towards sunlight. Low serum 25(OH)D level (12 ng/ml) in Japan is more common among inactive elderly (Lips, 2007). On the other hand, obese individuals have higher tendency to be vitamin D deficient compared to normal weight individuals (Foss, 2009). Increased storage of serum 25(OH)D in adipose tissue might explain the increase rates of vitamin D deficiency among obese individuals (Lenders, Feldman, Von Scheven, Merewood, Sweeney, Wilson et al., 2009).

1.5 Obesity

Apart from vitamin D deficiency, obesity is another public health condition that is common worldwide. Obesity had reached an epidemic state all around the world (Low, Chin, & Deurenberg-Yap, 2009). Almost 54.9% of the American adult population is either obese or overweight. Obesity is a well-established risk factor for cardiovascular diseases in the general population, which may lead to mortality and morbidity in developed and developing countries like Malaysia (Alwan, 2011). It was also identified as a major determinant of many other noncommunicable diseases such as musculoskeletal diseases, respiratory problems, certain types of cancers, type 2 diabetes mellitus and gallbladder disease. The decrease of 10% to 15% of body weight would reduce medical problems and health risk in 90% obese patients (Willett, Dietz, & Colditz, 1999). This is due to the improvement of their blood pressure, glucose tolerance, heart function and lipid profile that will decrease other complications.

In Malaysia, the number of obese individuals is increasing over time. According to the nationwide Third National Health and Morbidity Survey (NHMS III) which was conducted in 2006, 29.1% of the 33,055 adults were overweight (BMI 25.0-29.9 kg/m²). This study also showed 14.0% of the adult population as obese (BMI >29.9 kg/m²). The latest survey conducted by the Fourth National Health and Morbidity Survey (NHMS IV) found that there was an increase in percentage from 2006 to 2011 among the obese population; from 14.0% to 15.1% respectively.

Serum 25(OH)D level was found to be inversely associated with adiposity. Previous studies have shown that obese individuals tend to have lower serum 25(OH)D level than those with normal body weight. Obesity is associated with the decrease in bioavailability of cutaneously and dietary synthesis of vitamin D in the body. This is likely secondary to the sequestration of vitamin D into a larger pool of adipose tissue (Holick, 2006).

University

1.6 Rationale of the study

Although Malaysia is a country that is sunny throughout the year, the prevalence of vitamin D deficiency among Malaysian is high. There are not many local researchers who carry out the study on risk factors affecting serum 25(OH)D level.

Besides that, most of these kinds of studies only used the Body Mass Index (BMI) which provides a poor identification of adiposity compared to the direct measurements of body fatness like Bioelectrical Impedance Analyzer (BIA) and Dual-energy X-ray absorptiometry (DXA). Among the local studies, only one study was conducted on vitamin D, obesity and metabolic syndrome, but the researchers used BMI as an adiposity indicator and only limit the study to the Malay ethnic group (Moy & Bulgiba, 2011). The study by Nurbazlin et al. (2013) and Chin et al. (2014) used Bioelectrical Impedance Analyzer (BIA), but the association of vitamin D level and adiposity was not the researcher's main research focus (Nurbazlin, Chee, Rokiah, Tan, Chew, Siti Nusaibah et al., 2013) and they only limited the study to the male population (Chin, Ima-Nirwana, Ibrahim, Mohamed, & Wan Ngah, 2014).

Therefore, we aim to study the factors that contribute to serum 25(OH)D level and its association with different adiposity indicators among multi-ethnic teachers in Malaysia.

1.7 Conceptual Framework



1.8 Objectives

1.8.1 General Objective

To study the factors contributing to serum 25(OH)D level among multiethnic teachers and the association between adiposity and serum 25(OH)D level.

1.8.2 Specific Objectives

- To determine the prevalence of vitamin D deficiency among multi-ethnic teachers.
- 2) To determine the prevalence of adiposity among multi-ethnic teachers.
- 3) To describe the association between serum 25(OH)D level and adiposity.
- 4) To describe the association between serum 25(OH)D level and its risk factors (sex, ethnicity, age, medical history, levels of physical activity, sun exposure avoidance, parathyroid hormone (PTH), blood glucose and blood pressure).
- 5) To describe the contribution of risk factors on serum 25(OH)D level.

CHAPTER 2: LITERATURE REVIEW

2.1 Vitamin D

2.1.1 Characteristics and sources of vitamin D

Vitamin D is a steroid hormone with pleiotropic actions on most cells and tissues in the body. It is a fat soluble vitamin which can be ingested orally or formed endogenously by the skin after exposure to ultraviolet (UVB) light with a wavelength of 290 to 320 nanometers (Institute of Medicine, 2010). Vitamin D is the easiest way of nutrient and supplement that we can find and take as much as possible without cost, as more than 90% of vitamin D come from sunlight exposure (Holick, 2005). Most people meet at least some of their vitamin D needs through sunlight exposure (Cranney, Horsely, O'donnell, Weiler, Ooi, Atkison et al., 2007; Institute of Medicine, 2010). Seasons, length of day, smog and skin melanin content are among the factors that influence ultraviolet light exposure on vitamin D synthesis.

Some of the researchers suggested that approximately 5 to 30 minutes of sunlight exposure between 10am to 3pm at least twice a week without sunscreen would lead to a sufficient vitamin D synthesis (Holick, 2002, 2007). Meanwhile, some suggested that individuals with limited sun exposure need to take other sources of vitamin D in their diet or take vitamin D supplement to achieve an adequate level of vitamin D in their body (Wolpowitz & Gilchrest, 2006; Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney et al., 2011).

However, very few foods contain vitamin D naturally. Some examples are salmon, sardines, cod liver oil, egg yolk and others (**Figure 2.1**). Animal based foods can provide some vitamin D in the form of serum 25(OH)D; thus increasing the concentration of serum 25(OH)D level (Taylor, Patterson, Roseland, Wise, Merkel, Pehrsson et al., 2014). Study by Taylor et al. (2014) also showed that serum 25(OH)D content in beef, chicken, turkey and eggs can increase the estimated vitamin D level in the human body by two to eighteen times.

Selected Food Sources of Vitamin D		
Food	IUs per serving*	Percent DV**
Cod liver oil, 1 tablespoon	1,360	340
Swordfish, cooked, 3 ounces	566	142
Salmon (sockeye), cooked, 3 ounces	447	112
Tuna fish, canned in water, drained, 3 ounces	154	39
Orange juice fortified with vitamin D, 1 cup (check product labels, as amount of added vitamin D varies)	137	34
Milk, nonfat, reduced fat, and whole, vitamin D- fortified, 1 cup	115-124	29-31
Yogurt, fortified with 20% of the DV for vitamin D, 6 ounces (more heavily fortified yogurts provide more of the DV)	80	20
Margarine, fortified, 1 tablespoon	60	15
Sardines, canned in oil, drained, 2 sardines	46	12
Liver, beef, cooked, 3 ounces	42	11
Egg, 1 large (vitamin D is found in yolk)	41	10
Ready-to-eat cereal, fortified with 10% of the DV for vitamin D, 0.75-1 cup (more heavily fortified cereals might provide more of the DV)	40	10
Cheese, Swiss, 1 ounce	6	2

*IUs = International Units **DV= Daily Values

(Retrieved from National Institutes of Health, Office of Dietary Supplements)

Figure 2.1: Food sources rich with vitamin D

2.1.2 Different forms of vitamin D

Vitamin D can be either D_2 (ergocalciferol) or D_3 (cholecalciferol) (**Figure 2.2**). Vitamin D_2 is synthesized by plants and is not produced by the human body, while vitamin D_3 is made in large quantities in the skin when sunlight strikes bare skin. Vitamin D_3 can also be ingested from animal sources such as salmon, sardines, mackerel, eggs, cow's milk, beef liver and yogurt (Holick, 2005).

The two forms of vitamin D have traditionally been regarded as equivalent, based on their ability to cure rickets, but evidence suggests that vitamin D_3 is approximately three times more effective at maintaining serum concentrations because the binding protein has higher affinity to vitamin D_3 compared to D_2 . This allows vitamin D_3 to stay in the circulatory system longer, besides increasing the concentration to the sufficient level faster (National Institute of Health, 2009).



(Retrieved from www.britannica.com)

Figure 2.2: Different forms of vitamin D

2.1.3 Metabolism of vitamin D

Vitamin D₃ is made in the skin from 7-dehydrocholesterol under the influence of ultraviolet (UV) light. Vitamin D₂ (ergocalciferol) is derived from the plant sterol. Both forms of vitamin D are metabolized first to 25-hydroxyvitamin D (25(OH)D) (**Figure 2.3**), then to hormonal form 1,25-dihydroxyvitamin D (1,25(OH)₂D). The active form of vitamin D, which is 1,25(OH)₂D, is produced by the enzyme 25(OH)D-1 α -hydroxylase. This enzyme is regulated by Parathyroid Hormone (PTH) level and serum phosphorus.

When exposed to sunlight, the precursor provitamin D, 7-dehydrocholesterol which is in the epidermis and dermis will absorb ultraviolet light and transform into pre-vitamin D₃ (precholecalciferol) (Holick, 1981). It will be bound to the vitamin D binding protein (DBP) before it travels to the liver and be metabolized in the liver to 25-hydroxyvitamin D₃ by enzyme, 25-hydroxylase. 25-hydroxyvitamin D₃ will be converted to its active form, 1,25-dihydroxyvitamin D₃ by α_1 -hydroxylase in the kidney. The active form of vitamin D will increase the intestinal calcium and phosphorus absorption, besides inducing preosteoclasts to mature osteoclasts (Holick, 2012). Besides that, the active form of vitamin D can also assist in the stimulation of the insulin secretion in the beta islet cells of the pancreas, inhibit parathyroid hormone secretion and downregulation of renin production in the kidney (Holick, 2007).



(Hollis & Wagner, 2006)


2.1.4 Markers to determine vitamin D status in human

The circulating concentration of 25-hydroxyvitamin D (25(OH)D) is considered as a good marker or indicator to determine vitamin D status, compared to 1,25-dihydroxyvitamin D (1,25(OH)D₂), as it represents the cumulative effects of the dietary intake of vitamin D and sunlight exposure (Holick, 2008). Kovacs (2012) also proved that serum 25(OH)D has long circulating half-life of 14 to 20 days, making it suitable to measure the vitamin D status in human body.

In contrast to 25(OH)D, $1,25(OH)D_2$ is generally not a good indicator of vitamin D status, due to its short half-life of 15 hours and level of serum concentrations which is closely regulated by parathyroid hormone (PTH), phosphate and calcium (Jones, 2008). $1,25(OH)D_2$ levels do not typically decrease until vitamin D deficiency is severe (Cranney, Horsely, O'donnell, Weiler, Ooi, Atkison et al., 2007; Holick, 2007).

2.1.5 Definition of vitamin D deficiency

There is a considerable amount of discussion for the cut-off point in defining vitamin D deficiency. Based on its review of vitamin D needs among adults, a committee of the US Endocrinology Society Clinical Practice concluded that individuals at risk of vitamin D deficiency are at serum 25(OH)D level <20 ng/ml while potentially being at risk of insufficiency at levels ranging from 21-40 ng/ml. Practically, all individuals are sufficient with vitamin D at level >40 ng/ml (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney et al., 2011). This guideline was used in many studies as it does not just take into account for bone health only, but also for other cardiovascular diseases.

The above is different with the guideline by the Institute of Medicine (Institute of Medicine, 2010) in which individuals with <10 ng/ml serum 25(OH)D level are considered as vitamin D deficient while those with serum 25(OH)D level in the range between 10-20 ng/ml are classified as vitamin D insufficient. Meanwhile, individuals with \geq 20 ng/ml had sufficient vitamin D level in their bodies.

The definition of vitamin D deficiency based on the International Osteoporosis Foundation & DSM Nutritional Product (2012) is slightly different from other institutions, since the definition of serum 25(OH)D level are categorized into four: which are deficiency, insufficiency, sub-optimal and optimal. Individuals are considered as vitamin D deficient when serum 25(OH)D level is <10 ng/ml. The different definitions of vitamin D deficiency are shown in **Figure 2.4**.

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US Endocrine Society Clinical Practice Guideline (2011)

25	50	75	100	125	pmol/L
10	20	30	40	50	ng/ml
Deficien	t		ient	Sufficient	

International Osteoporosis Foundation & DSM Nutritional Products (2012)

25	50	75	100	125	pmol/L	
10	20	30	40	50	ng/ml	
Deficient Insufficiency Suboptimal Optimal						

Institute of Medicine (2011)

30	50	75	100	125	pmol/L
10	20	30	40	50	ng/ml
Deficient	> Insuffic		-		

Figure 2.4: Different definitions of vitamin D deficiency

2.1.6 Methods to measure serum 25-hydroxyvitamin D (25(OH)D)

2.1.6.1 Manual immunoassays

a) Diasorin Radioimmunoassay (RIA)

The DiaSorin 25-hydroxyvitamin D RIA assay is a two-step procedure involving a rapid extraction of 25(OH)D and other hydroxylated metabolites from serum and plasma, followed by a competitive RIA procedure using antibody with specificity for 25(OH)D (Wallace, Gibson, De La Hunty, Lamberg-Allardt, & Ashwell, 2010). The first step involves a rapid extraction of 25(OH)D and other hydroxylated metabolites from serum or plasma with acetonitrile. Following extraction, the treated sample is then assayed by competitive RIA using an antibody with specificity to 25(OH)D. The sample, antibody and tracer are incubated for 90 minutes at 20 to 25 °C. Phase separation is accomplished after 20 minutes of incubation at 20 to 25 °C with a second antibody precipitating complex. To reduce non-specific binding, buffer is added after this incubation, prior to centrifugation.

The RIA kit, developed by DiaSorin (Saluggia, Italy) is the most common method used by many reference laboratories (Hollis, 2010). This method has been used to establish reference ranges during the past decade. In comparison with the High Performance Liquid Chromatography (HPLC), Hollis (2000) reported that the Diasorin RIA recovered 91–100% of both 25(OH)D₂ and 25(OH)D₃. However, other studies suggested that Diasorin RIA underestimated 25(OH)D₂ relative to HPLC (Glendenning, Noble, Taranto, Musk, Mcguiness, Goldswain et al., 2003; Glendenning, Taranto, Noble, Musk, Hammond, Goldswain et al., 2006).

b) Immuno Diagnostic System (IDS) Enzyme Immunoassay (EIA)

The IDS 25(OH)D EIA kit is an enzyme immunoassay for the quantitation of 25(OH)D and other hydroxylated metabolites in plasma or serum (Wallace, Gibson, De La Hunty, Lamberg-Allardt, & Ashwell, 2010). Calibrators, controls and samples are diluted with 25(OH)D labelled with biotin. A propriety buffer reagent is used for dissociating 25(OH)D from its binding proteins. The diluted samples are incubated in microtitre wells, which are coated with a highly specific sheep 25(OH)D antibody for two hours at room temperature before aspiration and washing. Enzyme (horseradish peroxidase) labelled as avidin, is added and it binds selectively to complexed biotin. By following a further wash step, colour is developed using a chromogenic substrate (TMB). The reaction is stopped by the addition of hydrochloric acid and the absorbance read in a microtitre plate reader; colour intensity developed being inversely proportional to the concentration of 25(OH)D.

The advantages of using this method are it is technically simple and also inexpensive. However, this techniques might underestimate the $25(OH)D_2$ (Wallace, Gibson, De La Hunty, Lamberg-Allardt, & Ashwell, 2010).

2.1.6.2 Automated immunoassays

a) Diasorin Liaison Automated Immunoassay

This method is a direct, competitive chemiluminescence immunoassay (CLIA) on an automated platform (Wallace, Gibson, De La Hunty, Lamberg-Allardt, & Ashwell, 2010). Specific antibody to vitamin D is used for coating magnetic particles (solid phase) and vitamin D is linked to an isoluminol derivative. During the incubation, 25(OH)D is dissociated from its binding protein and competes with labelled vitamin D for binding sites on the antibody. After the incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25(OH)D present in calibrators, controls or samples.

According to Vitamin D External Quality Assessment Scheme (DEQAS), recoveries using Diasorin Liaison methods are good; 81% for $25(OH)D_3$ and 89% for $25(OH)D_2$ at a concentration of 14.4 ng/ml (Carter, Jones, & Berry, 2007). This method underwent a reformulation and had been replaced by DiaSorin Liaison Total Assay in 2008.

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b) Diasorin Liaison Total Automated Immunoassay

The Liaison Total 25(OH)D assay is a direct competitive chemiluminescence immunoassay (CLIA) for a quantitative determination of the total 25(OH)D in serum or plasma on an automated platform (Wallace, Gibson, De La Hunty, Lamberg-Allardt, & Ashwell, 2010). This method is a reformulation of the Diasorin Liaison method as mentioned before. The same antibody is used, but now in a two-step incubation procedure. During the first incubation, 25(OH)D is dissociated from its binding protein and binds to the specific antibody on the solid phase. After 10 minutes, the tracer (vitamin D linked to an isoluminol derivative) is added. After the second 10 minutes incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25(OH)D present in calibrators, controls or samples.

Diasorin Liaison Total is currently the most popular method within DEQAS (Roth, Schmidt-Gayk, Weber, & Niederau, 2008). It is extensively used and it is a technically simple technique with high throughput. However, the limitation includes it being susceptible to matrix effects (Wallace, Gibson, De La Hunty, Lamberg-Allardt, & Ashwell, 2010). Matrix effects are known to be a problem with immunoassays, which can lead to a spuriously high result. Serum 25(OH)D cannot be accurately measured, unless it is released from its specific binding protein.

c) Electro-chemiluminescence Immunoassay (ECLIA)

ECLIA is a Roche' technology developed in 2008. It is a competitive immunoassay format used based on the streptavidin–biotin technology (Wallace, Gibson, De La Hunty, Lamberg-Allardt, & Ashwell, 2010). The assay employs a polyclonal sheep antibody against $25(OH)D_3$, which is ruthenium labelled. The vitamin D in the sample competes for binding with biotinylated 25(OH)D antigen which is bound to streptavidin coated microparticles. The test is intended for use on Elecsys and Cobas E automated immunoassay analyzers.

Based on this technology and combined with its well-designed, specific and sensitive immunoassays, ECLIA delivers reliable results. A study by Leino et al. (2008) proved that this method had good overall agreement with results determined by LC–MS/MS and RIA, although a large between-method variation was observed in individual samples.

d) IDS iSYS Automated Immunoassay

This method was introduced in early 2009. The assay is based on chemiluminescence technology performed on an automated platform (Wallace, Gibson, De La Hunty, Lamberg-Allardt, & Ashwell, 2010). Samples are subjected to a pre-treatment step to denature the vitamin D binding protein. The treated samples are then neutralised in an assay buffer and anti-25(OH)D antibody labelled with acridinium is added. Following an incubation step, magnetic particles linked to 25(OH)D are added. After a further incubation step, the magnetic particles are "captured" using a magnet. After washing and adding trigger reagents, the light emitted by the acridinium label is inversely proportional to the concentration of 25(OH)D in the original sample.

Similar with other automated immunoassays, this method is technically simple with a high throughput. However, this method might underestimate the reading of $25(OH)D_2$.

2.1.6.3 Direct detection methods

a) High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) method can quantitate $25(OH)D_2$ and $25(OH)D_3$. It is available in a kit form (Hitachi High-Technologies Corporation Tokyo, Japan and Thermo Fisher Scientific, Sunnyvale, CA), in an effort to standardize the quality of the test and to make the assays more cost effective and less labor intensive. This method can be used to analyze food and biological samples.

An HPLC system (Spectra System, Thermo Scientific, USA) equipped with a UV detector (UV 2000) was used for chromatographic analysis. The separation of the analytes was performed on RP C18 analytical column Synergy hydro-RP 4,6x250 mm, 4.0 μ m (Phenomenex Inc., USA). The mobile phase consisted of 70% acetonitrile, 25% methanol, 5% water and used in isocratic elution mode with a flow rate of 1.2 ml/min. Injections were done using a Rheodynetype 7125 six-port valve equipped with 20 μ l loop. The detection was carried out at 265 nm. Instrument control, data collection and quantitation were performed by the help of ChromQuest 4.2.34 software.

Even HPLC is considered as a gold standard to measure serum 25(OH)D level, but this method is expensive, requires large volumes of samples and a well-trained technician in order to run the machine.

b) Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC- MS/MS)

Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) is an attractive technique due to its ability to analyze the crude serum extracts within a short period of time to gain potentially high throughput. In LC-MS/MS, deproteinisation was achieved by acetonitrile (Chen, Mccoy, Schleicher, & Pfeiffer, 2008), acetonitrile plus sodium hydroxide (Tsugawa, Suhara, Kamao, & Okano, 2005), methanol (Knox, Harris, Calton, & Wallace, 2009) or a methanol and propanol mixture (Maunsell, Wright, & Rainbow, 2005).

Each method has its own advantages and disadvantages. In addition, although the precision of LC-MS/MS techniques has been approved, it tends to overestimate the reading concentration of serum 25(OH)D levels than its actual value. Consequently, lower prevalence of vitamin D insufficiency was reported by this technique. Besides, this method tends to be labor intensive, technically difficult and expensive (Wallace, Gibson, De La Hunty, Lamberg-Allardt, & Ashwell, 2010).

2.1.6.4 Summary of all methods

There are various methodologies available for the measurement of serum 25(OH)D level in both routine clinical and research studies. It is clear that there are advantages and limitations in each method.

The acceptable reference methods for vitamin D analysis are LC- MS/MS and HPLC, which can measure both vitamin D_2 and vitamin D_3 separately (Hollis, 2010). The Nutritional Laboratory at the Centers for Disease Control and Prevention (CDC) and the National Laboratory in the United Kingdom are using these methods for vitamin D and nutrition surveys analysis, mainly due to its potential to differentiate different forms of vitamin D in plasma in all ages of individuals (Chen, Mccoy, Schleicher, & Pfeiffer, 2008; Hunty, Wallace, Gibson, Viljakainen, Lamberg-Allardt, & Ashwell, 2010).

However, the measurement of serum 25(OH)D by immunoassays would remain the method of choice among many researchers for the reason of speed, convenience, cost, turnaround and one of the appropriate methods to be used in laboratories which requires higher throughput (Wootton, 2005; Hollis & Horst, 2007). When used in the right context, immunoassays are valuable tools for the clinician and researchers. However, we cannot expect the researchers to perform at the level of the HPLC or LC-MS/MS assays which do require more expensive equipment and trained staff (Hollis & Horst, 2007).

2.2 Epidemiology of vitamin D deficiency

2.2.1 Prevalence of vitamin D deficiency in European countries

Vitamin D deficiency affects over one billion people globally and people from all races in various age groups (Holick, 2005). **Figure 2.5** shows the level of vitamin D among the adult population (>18 years) globally. Vitamin D status in Europe varies according to the difference in season, latitude, nutrition intake, behaviours toward sunshine and skin pigmentation (Meyer, Falch, Søgaard, & Haug, 2004; Kauppi, Impivaara, Mäki, Heliövaara, Marniemi, Montonen et al., 2009; Melhus, Snellman, Gedeborg, Byberg, Berglund, Mallmin et al., 2010). The level of serum 25(OH)D is higher in Northern and Western Europe, compared to Southern and Eastern Europe respectively (Van Schoor & Lips, 2011). The high serum 25(OH)D levels in Sweden and Norway are most probably due to a high intake of cod liver oil and fatty fish (Melhus, Snellman, Gedeborg, Byberg, Berglund, Mallmin et al., 2010) whereas low serum 25(OH)D levels in Italy, Greece and Spain may be due to sunshine avoiding behaviour and skin pigmentation (Hossein-Nezhad & Holick, 2013).



Figure 2.5: Level of vitamin D on adult population (>18 years) globally

There is a high variation of serum 25(OH)D level in the Middle-East countries (Mishal, 2001; Hashemipour, Larijani, Adibi, Javadi, Sedaghat, Pajouhi et al., 2004; Atli, Gullu, Uysal, & Erdogan, 2005). A study among 1210 men and women in Iran between 20 and 69 years showed that the mean serum 25(OH)D was 8.24 ng/ml in which the prevalence of vitamin D deficiency was very high (Hashemipour, Larijani, Adibi, Javadi, Sedaghat, Pajouhi et al., 2004). The lowest serum 25(OH)D was seen in a study among older individuals in Saudi Arabia (Sedrani, Elidrissy, & El Arabi, 1983). Studies in Jordan and Turkey among women showed a strong relationship between serum 25(OH)D level decreased from women with western clothing to women with hijab and completely veiled women with *niqab*. Males had higher serum 25(OH)D level compared to females in these countries.

Many studies examined the vitamin D status in North-America (including Canada and Mexico) (Lappe, Davies, Travers-Gustafson, & Heaney, 2006; Egan, Signorello, Munro, Hargreaves, Hollis, & Blot, 2008; Orwoll, Nielson, Marshall, Lambert, Holton, Hoffman et al., 2009). The mean serum 25(OH)D level in 4495 individuals were 20.1 ng/ml among men and 19.8 ng/ml among women in the United States (Forrest & Stuhldreher, 2011). Interestingly, an earlier NHANES study showed that in 1998 to 1994, the mean for 25(OH)D level was 30 ng/ml while in 2001 to 2004, the mean serum of 25(OH)D level decreased to 24 ng/ml. These results indicated a decrease in the mean serum 25(OH)D level in the US population. In a representative sample of the Canadian population, the mean serum 25(OH)D level at the ages of 20 to 39, 40 to 59 and 60 to 79 years were 26.0, 26.6 and 28.8 ng/ml respectively (Langlois, Green-

Finestone, Little, Hidiroglou, & Whiting, 2010). A study among healthy middleaged men and women showed that 29% of them had serum 25(OH)D level <20 ng/ml (Brock, Huang, Fraser, Ke, Tseng, Stolzenberg-Solomon et al., 2010).

Only a few studies on vitamin D status were carried out in South- America such as Argentina, Brazil and Chile (Oliveri, Plantalech, Bagur, Wittich, Rovai, Pusiol et al., 2004; Saraiva, Cendoroglo, Ramos, Araujo, Vieira, Kunii et al., 2005; González, Alvarado, Rojas, Navarrete, Velásquez, & Arteaga, 2007). A study in Argentina showed that a clear North-South gradient was observed with a higher vitamin D level near the equator (Oliveri, Plantalech, Bagur, Wittich, Rovai, Pusiol et al., 2004), while a study among postmenopausal women in Chile showed that lower serum 25(OH)D level were observed in postmenopausal as compared with premenopausal women (González, Alvarado, Rojas, Navarrete, Velásquez, & Arteaga, 2007). The observed mean serum 25(OH)D level in postmenopausal women in Chile was very similar to the mean serum 25(OH)D level in independently living elderly in Brazil (Saraiva, Cendoroglo, Ramos, Araujo, Vieira, Kunii et al., 2005).

Meanwhile, studies among the African populations such as in Tanzania showed adequate and higher serum 25(OH)D level (Aspray, Yan, & Prentice, 2005; Haarburger, Hoffman, Erasmus, & Pillay, 2009). However, a lower serum 25(OH)D level was reported in Tunisia's women with veil (14 ng/ml), compared to those without veil (17.2 ng/ml) (Meddeb, Sahli, Chahed, Abdelmoula, Feki, Salah et al., 2005).

2.2.2 Prevalence of vitamin D deficiency in Asian countries

Studies on vitamin D status among Asian population also varied due to the difference in lifestyle behaviors, clothing, belief and latitudes. Several studies have demonstrated low serum 25(OH)D level (<30 ng/ml) in populations across India (Arya, Bhambri, Godbole, & Mithal, 2004; Vupputuri, Goswami, Gupta, Ray, Tandon, & Kumar, 2006; Zargar, Ahmad, Masoodi, Wani, Bashir, Laway et al., 2007). In North India (27°N), 78% and 84% of healthy hospital staff (Harinarayan, Ramalakshmi, Prasad, Sudhakar, Srinivasarao, Sarma et al., 2007) and pregnant women (Sachan, Gupta, Das, Agarwal, Awasthi, & Bhatia, 2005) were found to have hypovitaminosis D (<20 ng/ml) respectively. In South India (13°N), low serum 25(OH)D level (<30 ng/ml) were equally prevalent among all different population groups (Harinarayan, 2005; Harinarayan, Ramalakshmi, Prasad, Sudhakar, Srinivasarao, Sarma et al., 2007).

In Bangladesh (24°N), low serum 25(OH)D level (<30 ng/ml) is common in women regardless of age, clothing and lifestyle (Islam, Akhtaruzzaman, & Lamberg-Allardt, 2006). A total of 38% vitamin D deficient Bangladeshi women were from high income group and 50% in women from low income groups (Islam, Lamberg-Allardt, Karkkainen, Outila, Salamatullah, & Shamim, 2002). In Sri Lanka (7°N), 40.5% of healthy females had serum 25(OH)D level less than 20 ng/ml.

High prevalence of vitamin D deficiency (<30 ng/ml) in Southeast or East Asia can be explained by skin pigmentation, traditional clothing, limited outdoor activity and air pollution. Vitamin D status of the population in Southeast Asian countries has received relatively less attention. Prevalence of vitamin D deficiency (<30 ng/ml) in postmenopausal women were 47% in Thailand, 49% in Malaysia, 90% in Japan, and 92% in South Korea (Lim, Kung, Sompongse, Soontrapa, & Tsai, 2007). The study by Kim et al. (2010) among the middle-aged South Koreans showed that almost 64.5% of Korean females suffered from vitamin D deficiency (<30 ng/ml). A study in Bangkok, Thailand reported that 64.6% of adults had vitamin D deficiency (<30 ng/ml) (Chailurkit, Aekplakorn, & Ongphiphadhanakul, 2011).

A dual centred study in China showed that more than 90% of young women in Beijing and Hong Kong had serum 25(OH)D level <30 ng/ml. However, the prevalence of vitamin D deficiency among young women in North China (Beijing) was lower (18%), compared to the South China (Hong Kong) (40%). In Japan (35°N), vitamin D deficiency (12 ng/ml) is more common among inactive elderly and younger women, compared to older than 30 years old (Nakamura, 2002). Overall, the vitamin D status in Japan is relatively better than the regions in South Asia due to the high fish consumption (Ono, Suzuki, Kotake, Zhang, Nishiwaki-Yasuda, Ishiwata et al., 2005; Nakamura, 2006).

2.2.3 Prevalence of vitamin D deficiency in Malaysia

A study in 2004 among postmenopausal Malaysian women showed that 27% of Malay women suffered from hypovitaminosis D (20-40 ng/ml) while 71% of Malay women had vitamin D insufficiency (10-20 ng/ml) (Rahman, Chee, Yassin, & Chan, 2004). This was followed by the study among women of childbearing age living in Jakarta and Kuala Lumpur which reported that 60% of women had vitamin D deficiency (<20 ng/ml) (Green, Skeaf, Rockell, B.J., Lambert, Todd et al., 2008). Later, a study among Malay adults in Kuala Lumpur in 2011 showed that 70% of Malay adults suffered from vitamin D deficiency (<20 ng/ml) (Moy & Bulgiba, 2011).

In 2013, a study conducted among urban and rural women in Kuala Lumpur and Palong, Negeri Sembilan showed that 48% of women in urban areas had vitamin D deficiency (<12 ng/ml) (Nurbazlin, Chee, Rokiah, Tan, Chew, Nusaibah et al., 2013). The latest study in 2014 among Malay and Chinese men living in the Klang valley reported that the prevalence of vitamin D insufficiency (12-20 ng/ml) and vitamin D deficiency (<12 ng/ml) were 22.7% and 0.5% respectively (Chin, Ima-Nirwana, Ibrahim, Mohamed, & Wan Ngah, 2014). The prevalence of vitamin D deficiency among Malaysian was high.

2.3 Effects of vitamin D deficiency on health

2.3.1 Skeletal diseases

Vitamin D plays a very important role in skeletal metabolism (Lips, 2001) which assists in reducing the risk of osteoporosis fracture (Dawson-Hughes, Heaney, Holick, Lips, Meunier, & Vieth, 2004). Vitamin D has a dual benefit for the prevention of fractures in the elderly, besides having a benefit on bone density and muscle strength (Souberbielle, Body, Lappe, Plebani, Shoenfeld, Wang et al., 2010).

Vitamin D has been shown to improve muscle performance and reduce the risk of falling in community-dwelling as well as institutionalized elderly. A meta-analysis including 8 double- blind RCTs (n=2426) demonstrated that falling was significantly reduced by 13% in vitamin D supplemented individuals compared with those receiving calcium or placebo (Bischoff-Ferrari, Dawson-Hughes, Staehelin, Orav, Stuck, Theiler et al., 2009). Higher dose supplemental vitamin D (700 –1000 IU/day) reduced the relative risk of falls by 19%.

Prospective cohort studies provided consistent evidence that high circulating 25-hydroxyvitamin D (25(OH)D) level was associated with a reduced risk of cardio-metabolic related diseases (Muscogiuri, Sorice, Ajjan, Mezza, Pilz, Prioletta et al., 2012). Approximately 33% of women aged 60 to 70 years and 66% of those aged 80 years or older had osteoporosis (Larsen, Mosekilde, & Foldspang, 2004). It was estimated that 47% and 22% of women and men respectively with age 50 years and above will sustain an osteoporotic fracture in their remaining lifetime.

2.3.2 Non-skeletal diseases

2.3.2.1 Cardiovascular diseases

Cardiovascular disease (CVD) is a general term describing diseases related to heart, blood vessels or both. The evidence on the link between vitamin D deficiency and myocardial disease has recently been reviewed. A follow-up study using a nested case control study among 18,225 men found that there was an increased risk of myocardial infarction (MI) in those with \leq 15 ng/ml serum 25(OH)D level compared to those with \geq 30 ng/ml (RR = 2.42; 95% CI, 1.53– 3.84) (Giovannucci, Liu, Hollis, & Rimm, 2008). In a cohort study among 3258 patients undergoing coronary angiography, those with lowest serum 25(OH)D levels (<8 ng/ml) had significantly higher cardiovascular mortality, compared with those with higher levels of serum 25(OH)D (>28 ng/ml) after 8 years (Dobnig, Pilz, Scharnagl, Renner, Seelhorst, Wellnitz et al., 2008).

In the LUdwig-shafen RIsk and Cardiovascular health (LURIC) study, a prospective cohort comprising of 3300 patients referred to coronary angiography demonstrated a strong association between serum 25(OH)D level and several cardiovascular outcomes such as cardiovascular mortality (Dobnig, Pilz, Scharnagl, Renner, Seelhorst, Wellnitz et al., 2008), stroke, heart failure and sudden cardiac death (Pilz, Marz, Wellnitz, Seelhorst, Fahrleitner-Pammer, Dimai et al., 2008). The lowest risk was among those with the highest serum 25(OH)D level.

2.3.2.2 Hypertension

In addition to possible direct effects due to the presence of the vitamin D receptor (VDR) and 1-alpha hydroxylase enzyme in cardiomyocytes and other cells of the cardiovascular system (Tishkoff, Nibbelink, Holmberg, Dandu, & Simpson, 2008), vitamin D has significant effects on several cardiovascular risk factors. A previous study found a significant association between the lowest level of serum 25(OH)D (<14.8 to 20.4 ng/ml) and incidence of hypertension over seven to eight years (Pittas, Dawson-Hughes, Li, Van Dam, Willett, Manson et al., 2006).

In a small randomized control trial (RCT) within 8 weeks of supplementation with vitamin D (800 UI/day) and calcium was shown to be more effective in reducing systolic blood pressure compared to calcium alone (Pfeifer, Begerow, Minne, Nachtigall, & Hansen, 2001). A study by Rosen et al. (2012) also showed that low serum 25(OH)D level was related with the increased risk of hypertension.

2.3.2.3 Type 2 Diabetes

Vitamin D deficiency impairs insulin secretion of pancreatic β-cells and increases insulin resistance in target tissues, which play critical roles in type 2 diabetes development (Knekt, Laaksonen, Mattila, Härkänen, Marniemi, Heliövaara et al., 2008). In the Nurses' Health Study of more than 83,000 subjects who took at least 800 IU of vitamin D daily had a 33% lower risk in developing type II diabetes (Pittas, Dawson-Hughes, Li, Van Dam, Willett, Manson et al., 2006). A meta-analysis of observational studies confirmed the association of low serum 25(OH)D level with incidence of diabetes (OR, 0.82; 95% CI, 0.72–0.93) (Lichtenstein, Ferreira-Júnior, Sales, Aguiar, Fonseca, Sumita et al., 2013).

2.3.2.4 Neurological diseases

Vitamin D plays a vital role in the nervous system. Vitamin D receptors are also located within the brain, including the primary motor cortex. These receptors and the enzymes responsible for the initial hydroxylation of vitamin D have been found within the majority of the Central Nervous System (CNS). Low serum 25(OH)D level is associated with neurological diseases such as Alzheimer's disease, Parkinson's disease, depression and cognitive decline (Llewellyn, Lang, Langa, Muniz-Terrera, Phillips, Cherubini et al., 2010). The major benefits of vitamin D on the nervous system are it assists in neurotransmission, neuroprotection and neuroplasticity.

2.3.2.5 Immune-related diseases

Vitamin D also plays an important role in the immune system. It seems to act as an immunosuppressive agent when a macrophage is stimulated by an infective agents like tuberculosis, expression of vitamin D receptors and 1 α hydroxylase are upregulated. The circulating 25(OH)D bound to serum vitamin D-binding protein enters the macrophage in its free form before being converted to active 1,25(OH)₂D. Then, 1,25(OH)₂D enters the nucleus and increases the expression of cathelicidin, which eventually promotes innate immunity and induces destruction of the infective agent (Hewison, 2011).

Previous studies demonstrated the association between multiple sclerosis with the decrease in serum 25(OH)D level. A study in 2006 showed that patients with serum 25(OH)D level >40 ng/ml had 51% reduction in risk of having multiple sclerosis, compared to those with serum 25(OH)D level <30 ng/ml (Munger, Levin, Hollis, Howard, & Ascherio, 2006).

2.3.2.6 Cancers

Cancer is a disease characterized by abnormal cells that grow and invade the healthy cells in the body. There are many different types of cancer such as breast cancer, lung cancer, brain cancer, prostate cancer and others. 1,25(OH)₂D-mediated repression or activation of proto-oncogenes or tumour-suppression genes that are related to cell proliferation and differentiation had been observed in a variety of normal and tumour tissues, including the small and large intestines (Lamprecht & Lipkin, 2003). This genetic mechanism seems responsible for the anti-cancer properties observed for vitamin D. Besides, many experimental data show that calcitriol stimulates apoptosis and differentiation and inhibits angiogenesis and proliferation in tumour cells (Fleet, 2008).

Numerous association studies suggested that serum 25(OH)D level are inversely associated with the risk of many types of cancer. Furthermore, in some studies of patients with cancer, an association between low serum 25(OH)D level and poor prognosis had been observed (Goodwin, Ennis, Pritchard, Koo, & Hood, 2009; Tretli, Hernes, Berg, Hestvik, & Robsahm, 2009). Prospective and retrospective epidemiological studies indicate that low level of serum 25(OH)D are associated with the increased risk for the incident of prostate cancer as well as colorectal and breast cancer (Gorham, Garland, Garland, Grant, Mohr, Lipkin et al., 2007). Other observational studies and meta-analysis had reported a weak and inconsistent association between dietary vitamin D and serum 25(OH) with cancer risk (Yin, Grandi, Raum, Haug, Arndt, & Brenner, 2010).

2.4 Factors contributing to vitamin D deficiency

2.4.1 Environmental factors (Exposure to sunlight radiation)

Ultraviolet radiation B needs to pass through the earth's atmosphere and ozone before reaching the ground and being absorbed by the human skin. However, as it travels down to the earth, it can be absorbed, scattered or reflected by substances like aerosols, cloud matter and pollutant particles. This eventually will affect the amount of ultraviolet radiation B reaching the earth and influence the synthesis of vitamin D by the human skin.

Air pollution such as black carbon particles which is generated by the combustion of fossil fuels acts as an efficient absorber of the ultraviolet radiation B. This leads to the reduction of ultraviolet radiation for up to 5% in a typical urban environment (Highwood & Kinnersley, 2006). This might be the reason why Bangkok, the main city in Thailand, had higher vitamin D deficiency level than other regions (Chailurkit, Aekplakorn, & Ongphiphadhanakul, 2011).

Cloud is another factor that affects the exposure of sunlight. Cloud attenuates the ultraviolet radiation B. Thick clouds can reduce ultraviolet radiation B to as much as 1% of clear sky level. A cloud cover of higher than 5.5 octa reduces the amount of biologically effective ultraviolet for pre-vitamin D_3 production (Parisi, Turnbull, & Turner, 2007).

Besides that, latitude and season also affect the serum 25(OH)D level. At the latitude of Denmark (54-58°N), no cutaneous vitamin D production occurs from October to April (Webb, Kline, & Holick, 1988). Within that period, vitamin D level was maintained by oral vitamin D intake and in the stores of the vitamin D built up during the previous summer. In Denmark, there is no food fortification with vitamin D. However, half of their population take vitamin D supplementation either throughout the year or at least during the winter season (Andersen, Mølgaard, Skovgaard, Brot, Cashman, Chabros et al., 2005). However, as Malaysia is a sunny country throughout the year, the latitude and season of the year are not the risk factors that affect vitamin D level among the Malaysian population.

2.4.2 Skin pigmentation

The ability of vitamin D synthesis among individuals depends on the cutaneous factors such as skin pigmentation, duration and surface area of exposure, clothing and sun protection behaviour. Increase in melanin content will reduce the efficacy and conversion of 7-dehydrocholesterol to pre-vitamin D_3 (Tsiaras & Weinstock, 2011). Individuals with a darker skin colour had less serum 25(OH)D level due to a slower vitamin D synthesis, compared to those with lighter skin colour. However, individuals with lighter skin colour are susceptible to lower tanning ability (Sturm, 2002), sunburn exposure and skin cancer (Kumar, Muntner, Frederick, Susan, & Michal, 2009).

Many studies looked at the relationship between skin lightness and vitamin D by using different methods and types of measurements of the skin colour. The Food and Drug Administration (FDA) and American Academy of Dermatology introduced six skin categories known as the Fitzpatrick scale or the Fitzpatrick phototyping scale (**Figure 2.6**).

Ethnicity has a great influence on skin colour and vitamin D level. A study by Jalaludin et al. (2013) found that Chinese had the highest mean of serum 25(OH)D level (26.4 ± 6.6 ng/ml) followed by Malays (17.7 ± 6.8 ng/ml) and Indians (15.8 ± 5.3 ng/ml) (p<0.001). This could be due to the darker skin color of Indians (Fitzpatrick skin type VI) and Malays (types V and VI) compared to Chinese (types III and IV) (Sng, Koh, Siong, & Choo, 2009).



(Retrieved from *Fitzpatrick Scale Image*)



2.4.3 Physical activity

2.4.3.1 Definition of physical activity

Physical activity is any bodily movement produced by the contraction of skeletal muscles which results in energy expenditure beyond resting expenditure (Caspersen, Powell, & Christenson, 1985). It can be a multi-faceted, complex and broad range of behaviours that may encompass daily activities such as gardening, house-work, stair climbing; transportation physical activities such as walking, bicycling and active travel; occupation-related activities such as lifting, packing, climbing up stairs and walking; leisure-time activities such as sports, doing hobbies, recreation and exercise; or engagement in specific prescribed interventions (Dugdill & Stratton, 2007).

Physical activity is further categorised as low, moderate or vigorous in which these classifications help to figure out the quantity of activity recommended (Rahl, 2010). The amount of activity differs by individuals, depending on personal choices or the given person over time (Caspersen, Powell, & Christenson, 1985). The quantity and types of physical activities have physiological and metabolic consequences in each individuals (Jackson, 2004). However, a sufficient amount of physical activity is essential to enhance a lean body mass (Brown, Thomas, & Kotecki, 2002).

2.4.3.2 Measurement of physical activity using questionnaires

There are many different tools that can be used to measure the level of physical activities. This difference in measurements is widely acceptable by health care providers, epidemiology researchers, exercise professionals and policy makers (Dubbert, 2002). It is important to have an accurate and precise measurement of physical activity level to investigate the association of its trends with diseases (Warren, Ekelund, Besson, Mezzani, Geladas, & Vanhees, 2010).

However, assessing physical activity in a field based research is fraught with difficulties due to its complexity and multidimensionality (Lamonte & Ainsworth, 2001). There is no single "gold standard" that exists to judge which method gives the most accurate measurement of physical activity level (Ferrari, Friedenreich, & Matthews, 2007). As a result, the development and refinement of valid and reliable measurements or tools for the assessment of physical activity should be the research priority for the continued advancement in the field (Valanou, Bamia, & Trichopoulou, 2006).

The procedure to assess physical activity level should consider the potential use in epidemiologic studies with respect to four main criteria: 1) to be valid, in which the assessment tool should also measure what it claims to measure, 2) to be reliable, the instrument must yield consistent results when it is applied more than once under the same circumstances; when the instrument is reliable and valid, it is also accurate; 3) to be practical, the instrument must be justified to be cost-effective to both the investigator and the participants; 4) to be non-reactive, the instrument must not alter the population or the behaviour it seeks to measure (Laporte, Montoye, & Caspersen, 1985).

Self-reported physical activity questionnaire is the most commonly used method in the population level (Shephard, 2003). Questionnaires is a method of collecting information about individual's information, beliefs, knowledge, attitudes and behaviours which is always used in cross-sectional surveys within the epidemiological studies and clinical trials (Boynton & Greenhalgh, 2004). In the questionnaire's selection, it is important to consider its content, sensitivity, extent, reliability and validity. Existing recall surveys are different in terms of their period to recall which can be ranged from the last 24 hours, last week, last month or last year (Timperio, Salmon, Rosenberg, & Bull, 2004). Even the questionnaires are varied in different formats such as open-ended question, scaling, multiple choice questions, rankings and ratings, as well as delivery such as in-person, telephone, email, web and point-of-control (Connaway & Powell, 2010).

The benefits of using questionnaires are they do not influence the pattern of a regular physical activity and it allows the evaluation of many variables using the same tool. Apart from that, they are also cheap, non-invasive and easily distributed (Armstrong, Cairns, Green, Reeves, & Beral, 2011). Since they are low cost in terms of time and money, the information on physical activity from a large number of studies can conveniently be collected, thus it is one of the most popular methods in large scale epidemiological studies (Gray, 2013).

Currently, they are more than 40 questionnaires being developed to measure the levels of physical activities among the adult population (Warren, Ekelund, Besson, Mezzani, Geladas, & Vanhees, 2010). This includes the Minnesota Leisure-Time Activity Questionnaire (Taylor, Jacobs, Schucker, Knudsen, Leon, & Debacker, 1978), the Baecke Physical Activity Questionnaire (Baecke, Burema, & Frijters, 1982), the Seven-Day Physical Activity Recall (7DPAR) (Sallis, Haskell, Wood, Fortmann, Rogers, Blair et al., 1985) and the Godin Leisure-Time Exercise Questionnaire (Godin & Shephard, 1997). The decision to select the specific measure of physical activity's questionnaire is based on the research questions.

The International Physical Activity Questionnaire (IPAQ) is one of the commonly used questionnaires to measure the levels of physical activity. IPAQ is a standardized instrument designed to assess habitual practice of physical activities from different countries. It can be used for cross-national monitoring of physical activity and inactivity. This international study has been validated for use in collecting physical activity data among 18 to 65 year old adults in various countries.

The IPAQ has gained remarkable popularity due to its approach in measuring the frequency, duration type and intensity level of physical activities performed in the past 7 days by each individual. It is a comprehensive evaluation of physical activity that has provided a more sensitive discrimination between individuals of different activity levels and lends itself to sub-analyses based on type, intensity and duration of activity (Ekoé, Rewers, Williams, & Zimmet, 2008). The rationale behind the development of the IPAQ is that although the global public health burden of physical inactive lifestyle has been recognized, to date, the impact and prevalence of the dilemma have not been analysed in a systematic manner. In 1998 to 1999, an initial pilot testing was carried out in 14 centres across 12 countries in six continents, using IPAQ which was developed in a standardized method. The 12 countries involved were Australia, Finland, Canada, Sweden, Netherlands, Guatemala, Brazil, South Africa, Japan, United States, United Kingdom and Portugal. The IPAQ had been vigorously tested for reliability and validity for the adults among different countries and populations in 2000 (Booth, Ainsworth, Pratt, Ekelund, Yngve, Sallis et al., 2003).

The advantage of IPAQ as compared to other physical activity questionnaires' is that it is designed to measure activities at multiple domains. Four domains of physical activity are addressed in IPAQ which are activities at the workplace, transportation, domestic care or household and leisure time, which later adds up to the total of physical activity. The assessment of physical activity in multiple domains is essential as this can assist in promoting effective interventions in countries that have different patterns of physical activities (Bouchard & Katzmarzyk, 2000).

The IPAQ is available in two different forms; a short version (9 items) and a long version (31 items), in which both involved a 7-day recall of the duration in different levels of physical activity. However, a short validated Malay IPAQ questionnaire was used in this study. The short form consisted of eight items providing information about the daily duration and weekly frequency of vigorous and moderate intensity activities, walking and inactivity (time spent sitting). The short form had been suggested to be used in population surveillance where the time constraint is an important concern as the short form is much more simple and practical than the long form (Maddison, Mhurchu, Jiang, Vander Hoorn, Rodgers, Lawes et al., 2007; Oyeyemi, Oyeyemi, Adegoke, Oyetoke, Aliyu, Aliyu et al., 2011).

2.4.3.3 Vitamin D and physical activity

Limiting outdoor activities can further reduce the amount of sun exposure and the formation of cutaneous vitamin D_3 , especially in the elderly population. Many studies among elderly population who are institutionalised (nursing home or residential care), hospitalised, housebound and those with greater disabilities have lower vitamin D level compared to healthy adults, due to their limited time spent outdoor (Lips, 2001; Rahman, Chee, Yassin, & Chan, 2004; Rockell, Skeaff, Williams, & Green, 2006; Van Dam, Snijder, Dekker, Stehouwer, Bouter, Heine et al., 2007; Van Der Mei, Ponsonby, Dwyer, Blizzard, Taylor, Kilpatrick et al., 2007).

A study by Kimlin et al. (2007) found that the mean serum 25(OH)D was significantly higher among individuals who usually spent one to two hours outdoor (27.7 ng/ml on weekdays; 24.7 ng/ml on weekends) compared to those who spent less than 15 minutes (17.1 ng/ml on weekdays; 13.2 ng/ml on weekends). A decrease in physical activities had been associated with a low vitamin D level in Europe and USA (Mithal, Wahl, Dawson-Hughes, Bonjour, Burckhard, Eisman et al., 2009). Besides that, low level of physical activity can also contribute to obesity and eventually cause vitamin D deficiency due to less availability of circulating serum 25(OH)D in the body.

2.4.4 Sun avoidance behavior

Sun avoidance behavior includes wearing a hat, using umbrella, wearing long sleeves, sunglasses and use of lotion or sunscreen. Findings from a research carried in Malaysia showed that clothing style is associated with low vitamin D level among females (Moy, 2011). Malay women have high prevalence of vitamin D deficiency compared to Chinese due to their dressing, as they cover most parts of their body. There was a negative correlation between sun avoidance score (the sum from the usage of sun block lotion, veil, cap/hat, wearing long sleeve, skirts, long pants, gloves and umbrella) and vitamin D level (r=-0.41, p<0.001) (Moy, 2011).

Similarly, individuals who covered most of their body parts in Turkey had higher prevalence of vitamin D deficiency (p<0.001) (Buyukuslu, Esin, Hizli, Sunal, Yigit, & Garipagaoglu, 2014). In the United States, individuals who were wearing long sleeves and staying in the shade were found to have significantly lower serum 25(OH)D level in whites, but not significant in Hispanics or blacks (William & Martin, 2011).
2.4.5 Age

Age is another factor that influences serum 25(OH)D level in human body. Vitamin D deficiency is common among the older population (Burnand, Sloutskis, Gianoli, Cornuz, Rickenbach, Paccaud et al., 1992). Older individuals are at risk of developing vitamin D deficiency due to low exposure to sunshine, decreased capacity of the older skin to synthesize vitamin D (Holick, Matsuoka, & Wortsman, 1989) and low dietary vitamin D intake (Lips, Van Ginkel, Jongen, Rubertus, Van Der Vijgh, & Netelenbos, 1987).

A study conducted by Wicherts et al. (2007) showed that individuals with serum 25(OH)D level <20 ng/ml were more often among the older population. A study by Rucker (2001) among the Canadian population showed that increasing age was associated with lower level of 25(OH)D and 1,25(OH)₂D. This might be due to several theories such as the decrease in the availability of 25(OH)D as a substrate (Bouillon, Auwerx, Lissens, & Pelemans, 1987), compounded by decreased renal function (Gallagher, Riggs, Eisman, Hamstra, Arnaud, & Deluca, 1979) and decreased in renal 1 α -hydroxylase activity with age (Slovik, Adams, Neer, Holick, & Potts Jr, 1981). Apart from that, the declining serum 25(OH)D level with age have been attributed to an impaired vitamin D absorption from the intestine (Clemens, Zhouf, Myles, Endres, & Lindsay, 1986) and the decline in the concentration of vitamin D's precursor which are normally stored in the skin (Maclaughlin & Holick, 1985).

2.4.6 Parathyroid Hormone (PTH)

Parathyroid Hormone (PTH) also affects serum 25(OH)D level. A low level of serum 25(OH)D and calcium exert a positive feedback on serum PTH to increase the level of calcium that eventually stimulates the conversion of 25(OH)D to vitamin D. It also assists the absorption of vitamin D from the gut. In the daily clinical situation, PTH measurements are used to identify the degree of hypovitaminosis D. PTH and 25(OH)D levels are inversely related. Hyperparathyroidism is commonly seen within the group of individuals with persistently low serum 25(OH)D level. However, PTH does not seem to be a sensitive marker of vitamin D deficiency among individuals (Andersen, Mølgaard, Skovgaard, Brot, Cashman, Chabros et al., 2005).

2.4.7 Dietary intake

Major food sources of calcium include dairy products, selected low-oxalate vegetables, legumes, nuts and fortified foods whereas primary sources for vitamin D include fortified dairy products, fatty fish and fortified foods. When environmental, social or physiological circumstances prevent adequate exposure to sunlight, dietary contribution becomes an important component to maintain serum 25(OH)D levels in the body. For countries with low consumption of vitamin D rich food such as fatty fish, the only alternative to increase their vitamin D level is to fortify specific food items or to use vitamin D supplements (Spiro & Buttriss, 2014).

In Spain, fish is the main dietary source, contributing 68% of dietary intake of vitamin D while eggs and cereals contributing 20% and 4% respectively (Spiro & Buttriss, 2014) which eventually affecting serum 25(OH)D level in the body. Besides that, Nakamura and colleagues found that fish consumption was positively associated with serum 25(OH)D level in elderly Japanese women (Nakamura, 2006). Individuals who ate fish frequently (four times per week) had significantly higher serum 25(OH)D by an average of 4 ng/ml than those who ate fish one to three times per week.

In adult Caucasian men and women in the United States, nutritional supplements contributed 30% and 40% respectively to the total vitamin D intake (Calvo, Whiting, & Barton, 2004), while among men and women in Norwegian (Jorde & Bønaa, 2000) and Britain (Henderson, Irving, Gregory, Bates, Prentice, Perks et al., 2003), nutritional supplements contributed 42% and 49%; 12% and 24% respectively.

A study by Rahman et al. (2004) among postmenopausal Malaysian women showed that food rich with vitamin D helped to maintain serum 25(OH)D level. Based on the dietary records, Chinese consumed fish like the Spanish mackerel, silver pomfret, anchovies, fish ball and fish cakes while the Malays consumed fish like the Indian mackerel, black pomfret, hardtail pomfret, sardines and anchovies. The consumption of eggs as well as milk and milk products which are also a source of vitamin D, was higher among the Malays, but was only significant (p<0.05) for the consumption of egg and its products (Rahman, Chee, Yassin, & Chan, 2004).

For healthy elderlies who are living in the community, the desirable serum 25(OH)D level of >30 ng/ml should be achieved by regular sunshine exposure, together with the consumption of fortified milk and other foods (Mckenna & Freaney, 1998). However, the overall patterns of dietary vitamin D intake (food and supplements) varied with gender, age and supplementation practices.

2.4.8 Obesity

2.4.8.1 Definition of obesity

Obesity is a clinical condition in which there is an above normal mass of adipose tissue, usually defined by the body mass index (BMI) \geq 30 kg/m². However, health risks seem to be higher at any level of BMI among Asian population. With that, World Health Organization (2004) proposed that BMI \geq 28.0 kg/m² as obese among the Asian population.

2.4.8.2 Prevalence of obesity in Europeans, Asians and Malaysians

Obesity is a major public health problem in the Western countries (Botella-Carretero, Alvarez-Blasco, Villafruela, Balsa, Vázquez, & Escobar-Morreale, 2007). The prevalence of obesity has significantly increased among these countries' population over the past 30 years and recent data estimated that nearly one-third of adults are obese (Holick & Chen, 2008).

Figure 2.7 shows the prevalence of obesity (\geq 30 kg/m²) among adults globally. In a systemic analysis of epidemiological studies from 199 countries (Finucane, Stevens, Cowan, Danaei, Lin, Paciorek et al., 2011), 1.46 billion adults worldwide were estimated to be overweight in 2008 while 502 million were obese. The number of overweight and obese individuals have increased from 921 million in 1980 to 2.1 billion in 2013 (Ng, Fleming, Robinson, Thomson, Graetz, Margono et al., 2014).





In the Global Burden of Disease Study, the estimated prevalence of obese individuals exceeded 50% among men in Tonga and women in Kuwait, Kiribati, Libya, Qatar, Tonga, and Samoa (Ng, Fleming, Robinson, Thomson, Graetz, Margono et al., 2014). In North America, the USA stands out for its high prevalence of obesity, with roughly one third of both men (31.6% [30.0-33.4]) and women (33.9% [31.8-35.7]) who were obese. The estimated prevalence of obesity was on average at 10% in Latin American countries and 17% in countries in North Africa and the Middle East.

More than 50% of the 693 million obese individuals in the world live in just ten countries (listed in order of number of obese individuals): USA, China, India, Russia, Brazil, Mexico, Egypt, Pakistan, Indonesia, and Germany (Ng, Fleming, Robinson, Thomson, Graetz, Margono et al., 2014). The largest increases in the rate of obesity were seen in Egypt, Saudi Arabia, Oman, Honduras, and Bahrain. The USA was among the top fifteen countries in terms of increases in obesity for both men and women.

In Asian adults, increases in the prevalence of obesity were seen in India, Nepal, Bangladesh and Malaysia, but not in China (Rokholm, Baker, & Sørensen, 2010). The prevalence of obese adults (BMI \geq 30 kg/m²) in most Asian countries is quite low, compared with developed countries such as the USA (Lin, Yen, Chen, Kao, Tzeng, Huang et al., 2003; Kim, Ahn, & Nam, 2005; Prentice, 2006). The highest prevalence of obesity in Asia was reported in Thailand in which 6.8% of adults were obese (Neal, Aekplakorn, Chaiyapong, Chariyalertsak, Kunanusont, Phoolcharoen et al., 2004). The lowest prevalence of obesity was reported in the less developed parts of Asia; 2.2% in India and 3.3% in the Philippines (Prentice, 2006). Further studies in 2013 showed that China and India had relatively low rates of obesity with 3.8% (3.5-4.3) and 5.0% (4.5-5.5) of Chinese men and women respectively, while 3.7% (3.3-4.1) and 4.2% (3.8-4.8) of Indian men and women respectively were considered as obese (Ng, Fleming, Robinson, Thomson, Graetz, Margono et al., 2014).

In Malaysia, there is an upward trend in the overweight and obesity rates over the years. According to the nationwide third National Health and Morbidity Survey (NHMS III) conducted in 2006, 29.1% of the 33,055 adult population was overweight (BMI 25.0-29.9 kg/m²) and 14.01% of the adult population are obese (BMI \geq 30.0 kg/m²). Findings by the fourth National Health and Morbidity Survey (NHMS IV) conducted in 2011 showed that the prevalence of obesity continued to increase from 4.4% in 1996 to 14.0% in 2006 and then increased more gradually to 15.1% in 2011.

2.4.8.3 Different measurements of adiposity

Clinicians and investigators have used a variety of measurements to measure adiposity, including the Body Mass Index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), Bioelectrical Impedance Analyzer (BIA) and others. However, anthropometric measurements such as Body Mass Index (BMI), waist circumference (WC), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) remain the most commonly used tools for assessing body composition, due to their low cost and easy handling (De Onis & Habicht, 1996).

BMI has been criticized as a measure of risk because it does not identify fat distribution (Mason, Craig, & Katzmarzyk, 2008). There is a growing body of evidence suggesting that abdominal adiposity is a more important risk factor for cardiovascular and metabolic diseases than overall adiposity (Janssen, Katzmarzyk, & Ross, 2004). Abdominal and overall obesity increased risk of type 2 diabetes mellitus, cardiovascular diseases, cancer and all causes of mortality (Koster, Leitzmann, Schatzkin, Mouw, Adams, Van Eijk et al., 2008; Chan, Malik, Jia, Kadowaki, Yajnik, Yoon et al., 2009; Kromhout, Geleijnse, Boer, & Verschuren, 2009), therefore the assessment of body adiposity is increasingly important in routine clinical practice.

a) Body Mass Index (BMI)

Body Mass Index (BMI) can be calculated as weight (kg) divided by height (m²). According to the World Health Organization (2004), obesity among the Asian populations is usually defined by a BMI of $\geq 28.0 \text{ kg/m}^2$. Individuals who are underweight are defined with a BMI of <17.5 kg/m² and a BMI in the range between 17.5 to 22.99 kg/m² is considered to be healthy/ normal. BMI in the range of 23.0 to 27.99 kg/m² is reported as overweight.

BMI is generally considered the best way to determine the healthy weight of an individual. Many studies were using BMI to measure obesity (Rahman, Chee, Yassin, & Chan, 2004; Moy & Bulgiba, 2011) as this is a simple, effective and quick tool to be applied on adults and children. It is a useful tool for the quick access of weight classification. However, BMI is not a direct measure of body fatness.

A WHO expert consultation reviewed scientific evidence which suggests that Asian populations have different associations between body fat percentage, BMI and health risks than European population. Asian population with a high risk of cardiovascular disease (CVD) and type 2 diabetes is substantial at the BMI that is lower than the existing WHO cut-off point for overweight (BMI ≥ 25 kg/m²) (World Health Organization, 2004).

b) Waist circumference

Waist circumference (WC) is a measure around the abdomen. It is the simplest and most common way to measure abdominal obesity. The waist is measured at the midpoint between the lower costal margin (lower rib) and the iliac crest (top of the pelvic bone) (**Figure 2.8**) (National Institute of Health, 1998). Waist circumference is one of the most practical tools to assess abdominal fat for chronic disease risk and during weight loss treatment. A high waist circumference or a greater level of abdominal fat is associated with an increased risk of high cholesterol, blood pressure, type 2 diabetes and heart disease.



(Retrieved from <u>www.presentdiabetes.com</u>)

Figure 2.8: Position to measure waist circumference

Majority of the previous studies confirmed that waist circumference (WC) is a better indicator of abdominal fatness compared to waist-to-hip ratio (WHR) (Vazquez, Duval, Jacobs, & Silventoinen, 2007) and BMI (Zhu, Heymsfield, Toyoshima, Wang, Pietrobelli, & Heshka, 2005) . WC is a surrogate marker for intra-abdominal adiposity and a strong predictor for cardiovascular risk factors among adolescents and children (Lee, Davis, Woolford, & Gurney, 2009; Johnson, Kuk, Mackenzie, Huang, Rosychuk, & Ball, 2010; Linghui, Na, & Jie, 2011).

There are difference between the cut-off points for waist circumference among Caucasians and Asians. The cut-off points of waist circumference among Caucasians adult population: 102 cm for men and 88 cm for women. These cutoff points were based on high sensitivity and specificity profiles against cut-off values of body mass index (BMI) \geq 30 kg/m² which has been considered as obese among Caucasians population (Lean, Han, & Morrison, 1995).

Meanwhile, the World Health Organization (WHO) Expert Committee on Obesity in Asian and Pacific populations suggested revised cut-off points of waist circumference among Asians adult population: 90 cm for men and 80 cm for women in identifying the individuals with abdominal. The cut-off point of waist circumference among Asians was based on the cut-off values of BMI \geq 28 kg/m² which has been considered as obese (World Health Organization, 2004).

c) Waist-to-height ratio

Waist-to-height ratio (WHtR) is defined as waist (cm) divided by height (cm). Studies showed that WHtR is significantly associated with the risk of obesity (Cox & Whichelow, 1996; Hsieh, Yoshinaga, Muto, Sakurai, & Kosaka, 2000). WHtR is more sensitive than waist circumference in different populations because of the negative correlation of height to certain metabolic risk factors (Henriksson, Lindblad, Ågren, Nilsson-Ehle, & Råstam, 2001), besides it being adjustable to different statures (Cox & Whichelow, 1996). However, there is no international cut-off points established for WHtR compared to waist circumference.

d) Bioelectrical Impedance Analyzer (BIA)

Bioelectrical impedance analysis (BIA) method is frequently used in research and clinical settings to measure body fat percentage, which is one of the cardiovascular and metabolic risk factors among adults (Sung, Lau, Yu, Lam, & Nelson, 2001; Sun, French, Martin, Younghusband, Green, Xie et al., 2005; Dehghan & Merchant, 2008; Barreira, Staiano, & Katzmarzyk, 2013). There are two types of BIA methods, which are foot-to-foot and direct segmental multifrequency method. In the foot-to-foot BIA method, there are four electrodes situated at each foot plate whereas the direct segmental multi-frequency method has eight electrodes on each foot plate and hand handle.

The foot-to-foot BIA (Figure 2.9) is more convenient in terms of simplicity and the measurements are also reproducible (Dehghan & Merchant, 2008). It can produce an acceptable quantification of body fat percentage (BF %) with no significant differences among adults (Sun, French, Martin, Younghusband, Green, Xie et al., 2005). This measurement is widely used in large scale population due to its availability, affordable price and simple technology to handle, especially when bringing it to the field during data collection (Linares, Ciangura, Bouillot, Coupaye, Declèves, Poitou et al., 2011; Bammann, Huybrechts, Vicente-Rodriguez, Easton, De Vriendt, Marild et al., 2013). Therefore, the foot-to-foot BIA is suitable for community screening in research studies, but caution should be taken while handling and interpreting the body fat percentage value because it may be dependent on several other factors such as gender, BMI and different ethnicity. However, the foot-to-foot BIA machine may not be as accurate as the direct segmental multi-frequency (DSM-BIA) (Figure 2.10). DSM-BIA measures impedance at five segments of the body including the whole body, both feet and hands by allowing the current and voltage to flow between feet and hand to give the reading of body fat percentage. The DSM-BIA has been compared with the whole body dual-energy X-ray absorptiometry (DXA) scan as an acceptable tool for the quantification of body fat percentage in adults. In low and middle income countries, there are increased demands in the usage of DSM-BIA in clinical settings such as adult obesity clinics, dietary and sport clinics. The DSM-BIA method has been proven to have high reliability and accuracy in healthy adults (Gibson, Holmes, Desautels, Edmonds, & Nuudi, 2008).

It is necessary and essential to measure the accurate body fat percentage rather than applying the conventional Body Mass Index (BMI) due to the increase trend in obesity, overweight and non-communicable diseases. The latest comparison study of portable foot-to-foot bioelectrical impedance scale to measure body fat percentage in Asian adults and children (2014) suggested that BIA is the more appropriate method that should be used in epidemiological studies (Sim, Su, Abd Majid, Nahar, & Jalaludin, 2014).



(Retrieved from <u>www.med-electronics.com</u>)

Figure 2.9: Foot-to foot BIA



(Retrieved from <u>www.med-electronics.com</u>)

Figure 2.10: Direct segmental multi-frequency (DSM-BIA)

e) Dual-emission x-ray absorptiometry (DXA- Scan)

Dual-emission x-ray absorptiometry (DXA scan) (**Figure 2.11**) is an enhanced form of x-ray technology and primarily used to evaluate bone mineral density. It can also be used to measure the total body composition and fat content with a high degree of accuracy (St-Onge, Wang, Shen, Wang, Allison, Heshka et al., 2004). The goal standard to measure the body fatness is by using DXA scan. It is one of the most accurate and precise methods. It is usually performed on the lower spine and hips.

DXA scan is a simple and quick tool which requires no anaesthesia. The amount of radiation used is extremely small. No radiation remains in the patient's body after carrying out the x-ray examination. In addition, this tool is the most accurate method used to diagnose osteoporosis and fracture risk. Even though DXA scan is a gold standard to measure body fat percentage, its use is limited to clinical settings as the machines are non-portable, bulky, expensive and requires technical expertise to handle. These tools are more often used in a clinical environment rather than in the research field.



(Retrieved from <u>www.med-electronics.com</u>)

Figure 2.11: Dual-energy x-ray absorptiometry (DXA)

2.4.8.4 Effects of obesity on health

a) Mortality

According to the recent National Institute of Health (NIH) statistics, obese individuals have a 50% to 100% increased risk of death from all causes compared to normal weight individuals (National Institute of Health, 1998). Mortality risk increases progressively with increasing BMI (Gu, He, Duan, Reynolds, Wu, Chen et al., 2006; Jee, Sull, Park, Lee, Ohrr, Guallar et al., 2006). Most of the increased risk of mortality related to overweight and obesity are due to cardiovascular diseases, kidney problems, diabetes and obesity related cancer deaths (Flegal, Graubard, Williamson, & Gail, 2007). Life expectancy of a moderately obese individual could be shortened by two to five years, while morbidly obese men could reduce their life expectancy by almost 13 years (Health & Services, 2009).

b) Cardiovascular diseases

The effects of obesity in public health are profound, as it has been shown to be a major risk factor for cardiovascular diseases (Lips, 2001; Dawson-Hughes, Heaney, Holick, Lips, Meunier, & Vieth, 2004). These include hypertension, Type 2 diabetes, impaired glucose tolerance and dyslipedaemia (Foss, 2009). Increased in obesity is also related to hypertension, oxidative stress, cardiac output, metabolic dysregulation, obstructive sleep apnea, increased in epicardial fat and myocardial fatty infiltration (Wong & Marwick, 2007).

Prospective studies documented that obesity is an independent predictor of clinical cardiovascular diseases including heart failure, coronary death, coronary heart disease and stroke in white and non-white populations (Kurth, Gaziano, Berger, Kase, Rexrode, Cook et al., 2002; Zhou, Wu, Yang, Li, Zhang, & Zhao, 2002; Suk, Sacco, Boden-Albala, Cheun, Pittman, Elkind et al., 2003). One of the earliest analyses was reported by Hubert et al. (1983) in the Framingham Heart Study. After 26 years of follow-up, they concluded that obesity was a significant independent predictor of cardiovascular diseases, including congestive heart failure, coronary death and coronary heart disease in both men and women; and stroke in women after the adjustment of risk factors. After 44 years of follow-up, Wilson et al. (2002) showed that cardiovascular disease risks (including angina, myocardial infarction or stroke) was higher among overweight obese men and obese women after the adjustment of age, smoking, high blood pressure, high cholesterol and diabetes.

c) Diabetes

Obesity and type 2 diabetes are likely to be the two greatest public health problems of the coming decades (Zimmet, Alberti, & Shaw, 2001). There is clear evidence of a strongly positive association between obesity and risk of diabetes. Insulin resistance is associated with obesity and promotes the development of type 2 diabetes (Guilherme, Virbasius, Puri, & Czech, 2008).

In the United States, half of those who were diagnosed with type 2 diabetes were obese (Leibson, Williamson, Melton, Palumbo, Smith, Ransom et al., 2001). In the Health Professionals follow-up study, 56% of the participants with type 2 diabetes were attributed to a weight gain of \geq 7 kg and a waist gain of \geq 2.5 cm (Koh-Banerjee, Wang, Hu, Spiegelman, Willett, & Rimm, 2004). The effects of substantial weight loss in obese individuals with type 2 diabetes were about the mechanisms underlying the relationship between obesity and type 2 diabetes (Taylor, 2008).

d) Cancers

Overweight or obesity is recognized as an important risk factor for some common cancers (Bianchini, Kaaks, & Vainio, 2002; Wiseman, 2008). Several meta-analyses (Kubo & Corley, 2006; Larsson & Wolk, 2007; Larsson, Orsini, & Wolk, 2007) confirmed that BMI is associated with cancer risks. A study in the United States estimated that overweight and obesity could account for 14% and 20% of all deaths of cancer in men and women respectively (Calle, Rodriguez, Walker-Thurmond, & Thun, 2003).

A recent systematic review found that obese men were at higher risk for oesophageal adenocarcinoma and thyroid, renal and colon cancers. Every additional 5 kg/m² in BMI increases a man's risk of oesophageal and colon cancer by 52% and 24% respectively (Renehan, Tyson, Egger, Heller, & Zwahlen, 2008). Among obese women, there were strong associations with gall bladders, renal, oesophageal adenocarcinoma and endometrial cancers. Every additional 5 kg/m² in BMI increases a women's risk of endometrial, gall bladder cancer by 59%. There was also a strong association of obesity with both premenopausal and postmenopausal breast cancers in women from the Asia Pacific region (Renehan, Tyson, Egger, Heller, & Zwahlen, 2008). An increase in every 5 kg/m² in BMI among postmenopausal women increases the risk of breast cancer by 12%.

e) Physical Impairment

Physical impairment is a limitation on a person's physical functioning, mobility or stamina. Physical constraints can make personal hygiene and cleanliness difficult. Obesity is strongly associated with daily activities related to mobility (Himes, 2000). There is a positive association between the level of obesity with musculoskeletal disorders, physical disability and osteoarthritis. Common examples of musculoskeletal pain experienced by obese people include lower limb pain, especially in the feet and knees following periods of standing and walking, with nagging lower back pains (Brown, Mishra, Kenardy, & Dobson, 2000).

There is also a strong relationship between obesity and the development of knee osteoarthritis, especially among women. The proportion of osteoarthritis attributable to obesity in middle aged women was 63% (Felson, Anderson, Naimark, Walker, & Meenan, 1988). Besides that, based on doctor's diagnosis, 31% of the arthritis adult patients were obese while 16% of them were non-obese (Hootman & Helmick, 2006). Modest weight loss in association with moderate physical activity improves the physical function, pain and performance among older obese adults with knee osteoarthritis (Messier, Loeser, Miller, Morgan, Rejeski, Sevick et al., 2004).

2.4.8.5 Association of vitamin D and adiposity

A mechanistic role of vitamin D in the regulation of adiposity and body weight has been suggested (Lips, 2001). The first evidence of the correlation between vitamin D and body fat was explained by Lumb et al., (1971). They hypothesized that after its absorption, vitamin D is sequestered and stored in tissues such as muscle and fat before it is released slowly into the blood circulation for biological use. Later, the same group demonstrated that by injecting radioactively labelled vitamin D₃, the highest concentration of biological activity and radioactivity vitamin D was seen in fat or adipose tissue (Lumb, Mawer, & Stanbury, 1971). More recently, Worstman et al. (2000) confirmed that the reduction in bioavailability of vitamin D₃ from cutaneous and dietary sources can contribute to obesity-associated vitamin D insufficiency, which was most likely due to vitamin D deposition in body fat compartments.

Circulating serum 25(OH)D levels are lower among obese individuals (BMI \geq 30 kg/m²) in many cross sectional studies (Arunabh, Pollack, Yeh, & Aloia, 2003; Parikh, Edelman, Uwaifo, Freedman, Semega-Janneh, Reynolds et al., 2004; Cheng, Massaro, Fox, Larson, Keyes, Mccabe et al., 2010). The most obvious explanations are the fat tissue may limit the bioavailability of vitamin D by reducing its entry into the circulation as vitamin D is a fat soluble vitamin which can be easily absorbed by the adipose tissue. Apart from that, fatter individuals receive less sun exposure because they spend less time outside or doing less outdoor physical activities (Wortsman, Matsuoka, Chen, Lu, & Holick, 2000; Stein, Flicker, Scherer, Paton, O'brien, Walton et al., 2001; Dowd & Stafford, 2010).

Another scientific reason to prove the significant correlation between vitamin D and adiposity in which the physiologic increase in parathyroid hormone levels in response to low serum 25(OH)D level is believed to increase the intracellular calcium in adipocytes which eventually leads to the increasing of lipogenesis and weight gain (Alemzadeh, Kichler, Babar, & Calhoun, 2008). Calcium modulates numerous physiologic functions including fat metabolism. Research has equated calcium and 1,250HD with lipogenesis and lipolysis (Tidwell & Valliant, 2011).

Based on the literature reviews, not many studies used the direct measure of body fatness such as Dual-emission X-ray (DXA) scan and Bioelectrical Impedance Analyzer (BIA). Most studies have largely been limited to BMI, waist-to-hip ratio and waist circumference (Arunabh, Pollack, Yeh, & Aloia, 2003; Alemzadeh, Kichler, Babar, & Calhoun, 2008). The goal standard to measure body fatness is by using the DXA scan. It is one of the most accurate and precise tools, particularly in thicker, heavy people. An epidemiologic study of adulthood in Western countries reported an inverse association between serum 25(OH)D level and body fat percentage measured by DXA scan (Dawson-Hughes, Heaney, Holick, Lips, Meunier, & Vieth, 2004). However, due to the costing and difficulty to handle when out in the field, some studies in Europe used the BIA instead of the DXA scan as a tool to measure body fatness of their samples, whereas it is very rare in Asia for using BIA. There were controversial results on the association of BMI, waist circumference and body fat percentage with serum 25(OH)D level. A study by Rodriguez et al. (2009) showed that the BMI of healthy women with higher vitamin D levels were smaller than those recorded in women with low vitamin D levels. A statistically significant negative correlation between BMI and serum 25(OH)D level among healthy adults of Caucasian and African Americans had also been reported (Parikh, Edelman, Uwaifo, Freedman, Semega-Janneh, Reynolds et al., 2004). These findings were different with the previous finding reported by Scragg et al. (1995) in a cross-sectional study among healthy population in New Zealand in which there was no correlation between serum 25(OH)D level and BMI.

Besides that, a study among middle-aged and elderly Chinese individuals showed that lower serum 25(OH)D level was inversely associated with larger waist circumference in both simple and multivariate linear regression analysis (Lu, Yu, Pan, Hu, Franco, Li et al., 2009). Study by Parikh et al. (2004) reported that there was a negative association between 25(OH)D level and body fat percentage (by using DXA) (r= -0.4; p <0.0001). Arunabh et al. (2003) found that serum 25(OH)D level was more strongly associated with body fat percentage, compared to BMI. However, recent studies showed contradicting findings (Nurbazlin, Chee, Rokiah, Tan, Chew, Siti Nusaibah et al., 2013; Chin, Ima-Nirwana, Ibrahim, Mohamed, & Wan Ngah, 2014).

Among all the studies conducted in Malaysia, only the studies carried out by Nurbazlin et al. (2013) and Chin et al. (2014) used BIA to relate the percentage of body fat with vitamin D status. However, that association was not the researchers' main research focus (Nurbazlin, Chee, Rokiah, Tan, Chew, Siti Nusaibah et al., 2013), and they only limited their samples to male population (Chin, Ima-Nirwana, Ibrahim, Mohamed, & Wan Ngah, 2014), whereas the rest of the studies used an indirect measurement which was BMI. A study conducted by Moy & Bulgiba (2011) reported the association between vitamin D and obesity. However, the researchers only focused among the Malay population rather than multi-ethnicity; besides, they were using BMI instead of BIA. With that, we recruited samples from multi-ethnic adults in our study and our aim was to determine the association between serum 25(OH)D level with adiposity (using various adiposity indicators) and its risk factors.

CHAPTER 3: METHODOLOGY

3.1 Study design, study population and sampling method

3.1.1 Study design

This was a cross-sectional study conducted in government secondary schools in Wilayah Persekutuan Kuala Lumpur (WPKL), from February 2013 to May 2013 (approximately 4 months). This study was a part of the CLUSTer Cohort Study of "Clustering of Lifestyle Risk Factors and Its Association with Stress on Health and Wellbeing among School Teachers in Malaysia (CLUSTer)" (Moy, Hoe, Hairi, Buckley, Wark, Koh et al., 2014).

3.1.2 Study setting

Government secondary schools (Sekolah Menengah Kebangsaan (SMK)) in Wilayah Persekutuan Kuala Lumpur (WPKL).

3.1.3 Study population

Teachers were selected because they were easily accessible during working hours and this occupation is contributed as one of the largest group of workers in the country (Ministry of Education, 2011). All permanent teachers in various ages working in the government secondary schools were invited to participate in our study. Pregnant teachers were excluded as their levels of adiposity and requirement for vitamin D may be different from those who were not pregnant.

A) Inclusion criteria

- All teachers in the government secondary schools in WPKL

B) Exclusion criteria

- Pregnant women

3.1.4 Sample size calculation

An open-epi computer software was used to calculate the sample size (www.openepi.com) (Figure 3.1). Referring to a study on the high prevalence of vitamin D insufficiency and its association with obesity and metabolic syndrome among Malay adults in Kuala Lumpur, Malaysia (Moy & Bulgiba, 2011), based on the 1.94 odds ratio (OR) of vitamin D deficiency among obese individuals, the required minimum sample size was 336. After adjusted to accommodate 30% of non-respondents; the required sample size was 437.



Two-sided significance level(1-alpha): Power(1-beta, % chance of detecting): Ratio of sample size, Unexposed/Exposed: Percent of Unexposed with Outcome: Percent of Exposed with Outcome: Odds Ratio: Risk/Prevalence Ratio:			95 80 1 30 45 1.9 1.5				
				Risk/Prevalence difference:			15
					Kelsey	Fleiss	Fleiss with CC
				Sample Size - Exposed	156	155	168
				Sample Size-Nonexposed	156	155	168
				Total sample size:	312	310	336

Figure 3.1: Sample size calculation

Complex sampling (two stage sampling) was used in our study. First, 50% out of all SMK in WPKL were randomly selected from each district (Bangsar, Sentul, Pudu and Keramat) by drawing lots (**Table 3.1**). Then, all teachers from the selected schools that fulfilled the inclusion criteria were invited to participate.

District	Total school	Number selected
PUDU	23	10
BANGSAR	23	10
SENTUL	21	10
KERAMAT	20	10
C		

Table 3.1: Total schools and number of selected schools in each district

3.2 Permission to carry out the study

3.2.1 Permission from the Ministry of Education, Educational Board and schools

Permission from the Ministry of Education (**Appendix A**) was obtained before getting the permission from the Educational Department of WPKL (**Appendix B**). After getting the approval, letters were sent to all selected schools at WPKL for a permission to carry out the study. Then, phone calls were made to set up the first appointment with the person in charge. During the first appointment, we explained the study and set up appointment dates to carry out data collection. Two to three days before the school visit for data collection, the schools were reminded via fax and phone calls. This was to ensure that all the participants (teachers) were aware of our visit for data collection and at the same time to remind them to fast at least eight hours before the blood test was carried out. Informed consent was obtained from all participants before the data collection (**Appendix C**).

3.2.2 Ethics clearance

Ethics clearance (**Appendix D**) was obtained from the University Malaya Medical Centre (UMMC) Ethics Committee (Reference Number: 950.1) that governs all the studies involving human subject within the Faculty of Medicine (FOM), University of Malaya.

3.3 Data collection

3.3.1 Questionnaires

Participants were enquired on their socio-demographic characteristics (**Appendix E**), sunlight exposure avoidance (**Appendix F**) and level of physical activity (**Appendix G**) using a self-administered questionnaire.

3.3.1.1 Socio-demographic characteristics

Socio-demographic characteristics on age, sex, ethnicity, marital status and medical history were enquired.

3.3.1.2 Sunlight exposure and avoidance

As more than 90% of the vitamin D is contributed from sunlight exposure, a questionnaire from Moy (2011) was used to describe the pattern of sun exposure and avoidance. The sun avoidance questionnaire was validated among 56 adults in another workplace. The sun avoidance score was negatively correlated with 25(OH)D (r= -0.41, p<0.001). Test-retest of the questionnaire was carried out over a two week period to test on the reliability. The mean (standard deviation) score for pre-tests and post-tests were 3.07 (1.08) and 2.94 (1.23) respectively. The correlation coefficient was 0.57 (p<0.001), the Cronbach alpha = 0.72 and the Intra-class correlation (ICC) was 0.72 (95% CI: 0.52, 0.84). These results showed that the questionnaire was valid and reliable.

Sun avoidance score was derived through the sum of usage of veil, sun block lotion, cap or hat, long sleeves shirt, gloves, long pants, long skirts and umbrella (max= 8, min= 0); while the sun exposure score was derived by multiplying the duration in minutes of sun exposure per day with number of days per week.

3.3.1.3 International Physical Activity Questionnaire (IPAQ- short form)

The IPAQ is available in two different forms; a short version (9 items) and a long version (31 items), in which both involved a 7-day recall of the duration in different levels of physical activity. Both versions are validated in the Malay language (Chu & Moy, 2015). The short version of IPAQ is a dimension-based instrument and found to be suitable for national and regional surveillance systems. Records on the time spent walking, in moderate and vigorous intensity activities and in sedentary activities were collected in the short version. The short form had been suggested to be used in the population surveillance in which time constraint is an important concern. This is because the short form is much more simple and practical to fill up than the long version (Maddison, Mhurchu, Jiang, Vander Hoorn, Rodgers, Lawes et al., 2007; Oyeyemi, Oyeyemi, Adegoke, Oyetoke, Aliyu, Aliyu et al., 2011). The short validated Malay IPAQ (IPAQ-M) was used in our study and it is available at the IPAQ website (*www.ipaq.ki.se*).

The scores of IPAQ were expressed in MET-minutes/week. For the analysis of IPAQ-M data, the following MET-values were used: walking= 3.3 METs, moderate physical activity= 4.0 METs, and vigorous physical activity= 8.0 METs. Then, MET-minutes per week (MET-min week⁻¹) was calculated as: minutes of activity/day x days per week x MET level. According to the IPAQ Research Committee (2005), given that the nature of non-normally distributed

energy expenditures were observed in many populations, it was suggested that the continuous indicator should be presented as median values and interquartile range (IQR), rather than means.

The outcome measures used from the IPAQ-M data were:

- Total physical activity score (MET-min week⁻¹) was defined by the sum of vigorous, moderate and walking physical activity scores. The sitting questions were developed as separate indicators and not as part of the summed physical activity score.
- MET-min week⁻¹ reported in vigorous and moderate physical activity and MET-min week⁻¹ spent on walking and sitting.
- 3) MET-min week⁻¹ reported in each domain (work, transportation, domestic, leisure-time domains) which is the summation or total scores for walking, moderate-intensity and vigorous-intensity within the specific domain.
- Three physical activity categories (low, moderate, high) were used to classify subjects based on their activity scores:
 - Low (<600 MET-min week⁻¹)
 - Moderate (600- 1499 MET-min week⁻¹)
 - High (1500- 2999 or >2999 MET-min week⁻¹)

3.3.2 Measurements

3.3.2.1 25-hydroxyvitamin D ((25(OH)D), Parathyroid Hormone (PTH) and blood glucose

Biochemical measurements on vitamin D, PTH and blood glucose were measured using fasting venous blood collected by trained paramedic staff. The blood samples were spun at 3000 revolutions per minute (RPM) for 10 minutes and 500ul/ml serum was aliquoted using a pipette into two different cryotubes of 1.8ml. One was used to measure vitamin D while the other one was used to measure PTH. Both cryotubes filled with serum were sent to the Clinical Diagnostic Laboratory (CDL), University of Malaya Medical Centre (UMMC) to perform a vitamin D test using a vitamin D reagent kit by a trained laboratorist. The steps or process were explained in **Figure 3.2**.

The circulating concentration of 25-hydroxyvitamin D (25(OH)D) is considered a good marker or indicator to determine the vitamin D status in our study. It is the most stable and plentiful metabolite of vitamin D in the human serum. It represents the cumulative effects of dietary intake of vitamin D and sunlight exposure (Holick, 2011). Kovacs (2012) also reported that 25(OH)D's serum half-life of 14 to 20 days makes it a suitable measure of vitamin D deficiency.


Figure 3.2: Steps in blood spinning

Electrochemiluminescence immunoassay (ECLIA) vitamin D_3 (25(OH)D) on Cobas E-411 analyzer (**Figure 3.3**) was used in our study as a method to test on serum 25(OH)D, as this is the only assay provided by the CDL, UMMC. The inter-assay coefficients of variation (CV) are 3.6% at 22.8 ng/ml and 3.0% at 68.2 ng/ml while the intra-assay CV are 3.5% at 22.8 ng/ml and 2.9% at 68.2 ng/ml. The measuring range for this kit is 4 to 100 ng/ml.

Based on the US Endocrinology Clinical Practice Guideline (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney et al., 2011), individuals with serum 25(OH)D level less than 20ng/ml are considered as vitamin D deficient.



(Retrieved from *Clinical Diagnostic Laboratory* (*CDL*) University of Malaya Medical Centre (UMMC))

Figure 3.3: Machine used to test serum 25(OH)D

The measurement of serum parathyroid hormone (PTH) level may assist to establish the diagnosis of vitamin D deficiency. PTH levels are often elevated in the individuals with vitamin D deficiency. Serum i-PTH was analyzed using ECLIA PTH on the Cobas E-411 analyzer. The inter-assay coefficient of variation (CV) are 6.2% at 2.14 pmol/L and 4.1% at 6.15 pmol/L while the intra-assay CV are 4.1% at 2.14 pmol/L and 2.2% at 6.15 pmol/L. The measuring range of this kit is 0.127 to 530 pmol/L.

Apart from that, approximately 10ml of blood sample was drawn from the participants after an overnight fast. Similarly, the analysis of blood glucose was also conducted in the Clinical Diagnostic Laboratory (CDL) of University of Malaya Medical Centre. Fasting plasma glucose level was measured using an automated analyzer (Siemens Healthcare Diagnostics Inc., Newark, USA). The intra-assay and inter-assay coefficients of variation for glucose assayed were 0.6% and 1.6% respectively.

3.3.2.2 Body fat percentage (BF %)

Bioelectrical Impedance Analyzer (BIA) is a rapid and portable method for body composition analysis and yields valid and reproducible results. The technique is based on the fact that lean tissues have a high water and electrolyte content, and thus provide a good electrical pathway (Vivian, 1996). It is available in two different methods which are foot-to foot method and direct segmental frequency method (DSM-BIA). Foot-to-foot BIA was used in our study (Model: TBF-300A Body Composition Analyzer) (**Figure 3.4**). This scale is inexpensive, easy to bring to the field, does not require special training and can provide a fairly good week-to-week comparison, as long as measurements are done on the same time of the day and not after exercise.



(Retrieved from <u>www.med-electronics.com</u>)

Figure 3.4: Tanita TBF-300A Body Composition Analyzer

Fat mass contains lower body water and thus it is a poor conductor of electrical signal. By inducing a low energy, high frequency, electrical signal (50 kHz, 500 microamp), a measurement of the baseline resistance to the flow of electrical current can be made. This current was passed through the anterior electrode on the scale platform and the voltage drop then was measured through the posterior electrode. The resistance measurement relates directly to the volume of the conductor which was used to determine the total body water, lean body mass and eventually body fatness. The percentage of body fat, as calculated by Tanita, is a highly researched proprietary formula combining impedance and weight measurements with height, age and gender information.

In adults, the prediction formula was BF % = 1.20 x BMI + 0.23 x age - 10.8 x sex-54 (R2 079, SEE = 41% BF %) (Paul et al.,1991). Internal and external cross-validation of the prediction formulas showed that they gave valid estimates of body fat in males and females at all ages. However among obese subjects, the prediction formulas may slightly overestimate the BF %.

An additional 0.5 kg was added into the BIA machine to factor in the weight of the participant's clothing. Participants were categorized as either being athletes or not, because different hydration levels have been observed in very athletic individuals as compared to standard or only moderately active adults (Battistini, Virgili, & Bedogini, 1994). In addition, they were also categorized based on different sex as males and females have physiological differences that must be accounted for when determining the body fat percentage. Generally, males carry more muscle compared to females. **Figure 3.5** shows the steps in using the BIA machine. Age and height were keyed in. Participants were asked to remove their shoes and socks before stepping on the machine. This was because any material which prevents clear skin contact with the electrode on the BIA machine may cause additional impedance that will eventually increase the percentage of body fat. The result was printed as shown in **Figure 3.6**.

University



Figure 3.5: Steps using BIA machine



Figure 3.6: Printed BIA slip result

Below is the range of body fat for adults based on the World Health Organization's (WHO) BMI Guidelines. However, we did not use this cut-off point of body fat percentage (BF %) (**Figure 3.7**) because there was no exact or gold standard of cut-off point established among the Asian population. Instead, tertile categories (tertile 1, tertile 2 and tertile 3) were used to categorize BF % (**Table 3.2**).



(Retrieved from the Manual Book of Tanita TBF-300A Body Composition Analyzer)

Figure 3.7: Body fat ranges among adult population by sex

 Table 3.2: Cut-off point of body fat percentage (%)

Category	Cut-off Point
Tertile 1	≤ 24.77
Tertile 2	24.78- 31.62
Tertile 3	≥ 31.63

3.3.2.3 Anthropometric measurements

Anthropometric measurements such as weight, height, waist and hip measurements were measured by using digital weighing scales (Model no- 813 SECA Robusta, Germany), stadiometer (Model no- 214 SECA, Germany) and circumference measurement tape (Model no- 201 SECA, Germany) respectively (**Figure 3.8**). All these equipment were calibrated regularly. The Body Mass Index (BMI) (kg/m²) was calculated using the formula of weight (kg) divided by height² (m²).

Height was measured in which the participants were asked to take off their shoes or socks. All hairstyles should allow the height measure to sit comfortably on their head. The participants were asked to stand on a levelled floor with feet parallel and pointing forwards. Each participant was standing unsupported and clear of any furniture which may impair the ultrasonic beam. The measure was placed on the participant's head before the reading. Weight was measured in which the participants were asked to take out shoes or socks or any heavy clothing that affect the weight's reading. The scales switched on the view finder should display [0.0]. Then, each participant was asked to step on the scales before the reading was taken.



Stadiometer, SECA 214 Circumference measurement tape, SECA 201

(Retrieved from <u>www.scalegalore.com/seca_sales.htm</u>)



Based on the classification of overweight and obese individuals in Asian countries by the World Health Organization (2004), underweight was defined with a BMI less than 17.5 kg/m² and BMI in the range of 17.5 to 22.99 kg/m² are healthy/ normal. BMI in the range of 23.0 to 27.99 kg/m² is considered as overweight whereas BMI above or equal to 28.0 kg/m² is considered as obese.

The waist was measured at the midpoint between the lower costal margin (lower rib) and the iliac crest (top of the pelvic bone) (National Institute of Health, 1998). Waist circumference provided information about the distribution of body fat, besides the risk of having metabolic syndrome. In men, those with more than 90 cm (waist circumference) have a high tendency to get metabolic syndrome while more than 80 cm for women have a high tendency to get metabolic syndrome (Misra & Khurana, 2008).

In our study, we did not compute both waist-to-hip ratio (WHR) and waist-toheight ratio (WHtR). The main reason was the majority of the studies showed a weak correlation between WHR and adiposity compared to waist circumference (WC). As for WHtR, the international cut-off point is still not well established yet as compared to the cut-off point of WC. Besides, both WHR and WHtR are in the ratio forms which are difficult to remember and interpret, compared to WC which is easier to measure and to be remembered by the participants.

3.3.2.4 Blood pressure

Blood pressure was measured using a clinical validated digital automatic blood pressure machine (OMRON HEM Model- 907, USA) (**Figure 3.9**) with the participants in a seated position and relaxed. This was to provide an assessment of the overall cardiovascular status of the participants. Any loose clothing covering the upper left arm was removed from the participants. The cuff was positioned securely 1 to 2 cm from the inside of the elbow joint before the START button was pressed.

Blood pressure was considered as high if the systolic/diastolic was more than 140/90 mmHg while blood pressure was considered low if systolic/diastolic was less than 90/40 mm/Hg. If the reading of the blood pressure was high, the measurement was repeated after three minutes. If the second reading was still high, the participant was asked to see a doctor for a further checkup. The ideal systolic/diastolic blood pressure is approximately 120/80 mm/Hg.



(Retrieved from <u>www.omron-healthcare.com</u>)

Figure 3.9: HBP-1300 Professional Blood Pressure Monitor

SUNACTION

3.4 Data analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). Weighted means \pm standard deviation (SD) and proportion were calculated for the descriptive data. Complex Sample Analysis was used in this study.

Schools' weightage was calculated by dividing the total schools with the total of participated schools within each district respectively (**Table 4.1**), while the teachers' weightage was calculated by dividing the total teachers in each school with the total teachers participated in the study. Eventually, the final weightage was calculated by multiplying schools' weightage and teachers' weightage (**Table 4.2**). The final weightage was used in the complex sample analysis. The weightage was used to correct unequal probabilities of the non-respondents.

An independent t-test, ANOVA, a simple linear regression and multiple linear regression analysis were conducted. Independent t-test and ANOVA were performed to analyse each categorical risk factor that influences the mean serum 25(OH)D level. A simple linear regression analysis was performed to determine the association of factors with serum 25(OH)D level, whereas a multiple linear regression analysis was performed to test the risk factors with serum 25(OH)D level after adjusting for other confounders. R^2 values were reported to compare the variation explained by each multivariate model. The significant level was preset at 0.05.



CHAPTER 4: RESULTS

4.1 Response rate, power of sample and sample weight

4.1.1 Response rate

There were a total of 858 teachers from 30 schools in all four districts; Pudu, Bangsar, Keramat and Sentul participated in this study. The response rate of returned questionnaires was 85.2%.

4.1.2 Power of sample

Using the OpenEpi stats calculator by comparing two means (**Figure 4.1**) with the Type 1 error of 5%, the mean serum 25(OH)D level among obese and normal weight participants were 16.56 ± 0.38 ng/ml and 19.20 ± 0.50 ng/ml respectively. With the existing sample size, the power of the study from the calculation was 100%.





Figure 4.1: Power of sample

4.1.3 Schools', teachers' and final weightage

The schools' weightage was calculated by dividing the total schools with the total of participated schools within each district respectively (**Table 4.1**).

Table 4.1: Schools' weightage by district

					Schools
	Total	Number	Number	Number	weightage
Districts	School	selected	declined	participated	by district
PUDU	23	10	1	9	2.556
BANGSAR	23	10	1	9	2.556
SENTUL	21	10	6	4	5.250
KERAMAT	20	10	2	8	2.500

The teachers' weightage from each selected schools were calculated in which the total number of teachers were divided by the participated teachers. Then final weightage was calculated by multiplying the teachers' weightage and the schools' weightage (**Table 4.2**).

	Total		Teachers	Schools	Final
School ID	Teachers	Participants	Weightage	Weightage	Weightage
CLUST/SP	124	44	2.819	2.556	7.203
CLUST/BBSP	83	38	2.184	2.556	5.583
CLUST/BTS	98	42	2.333	2.556	5.964
CLUST/SJ	87	31	2.806	5.250	14.734
CLUST/TSR	68	20	3.238	2.500	8.095
CLUST/SB	140	32	4.375	2.556	6.630
CLUST/MJ	107	20	5.350	5.250	19.163
CLUST/SS	153	12	12.75	2.556	22.365
CLUST/PA	56	34	1.697	2.500	4.242
CLUST/PMP	51	24	2.125	2.556	5.432
CLUST/WM	75	45	1.667	2.500	4.167
CLUST/CHE	117	22	5.318	2.556	7.901
CLUST/DTHO	105	6	17.50	2.500	26.667
CLUST/KB	158	39	4.051	5.250	14.135
CLUST/S5WM	126	32	3.938	2.500	6.875
CLUST/SH	78	30	2.600	2.556	6.646
CLUST/JI	120	24	5.000	5.250	18.375
CLUST/PB	31	21	1.476	2.556	3.773
CLUST/SBU	86	24	3.739	2.556	9.557
CLUST/AD	72	21	3.429	2.556	8.763
CLUST/DL	118	22	5.364	2.556	9.061
CLUST/DK	126	37	3.405	2.500	6.014
CLUST/TD	101	11	9.182	2.556	15.801
CLUST/MHJ	80	28	2.857	2.556	7.303
CLUST/LSB	33	13	2.538	2.556	6.488
CLUST/PP	81	53	1.558	2.556	3.981
CLUST/CJP	66	20	3.300	2.556	8.435
CLUST/TS	122	35	3.486	2.500	6.428
CLUST/PW	65	46	1.413	2.500	3.533
CLUST/SSJ	179	34	5.265	2.556	13.457

Table 4.2: Final weightage of each participated school

4.2.1 **Socio-demographic characteristics**

The age of participants ranged between 23 to 59 years with two third of them from the age group of 30 to 49 years. The majority of them were females, Malays and married. Less than 10% of our participants had medical history of diabetes, high blood pressure and heart disease. None of our participants had high cholesterol (Table 4.3).

Characteristic	N (Weighted %)		
General information			
Districts			
Bangsar	236 (28.2)		
Keramat	255 (21.1)		
Pudu	253 (24.8)		
Sentul	114 (25.9)		
Sex			
Male	77 (9.1)		
Female	781 (90.9)		
Age, years			
<30	137 (15.9)		
30-39	279 (31.6)		
40-49	293 (34.9)		
≥50	149 (17.6)		
Ethnicity			
Malay	660 (76.9)		
Chinese	125 (16.4)		
Indian	63 (8.3)		
Others	10 (0.4)		
Marital status [£]			
Single	103 (14.6)		
Married	611 (84.0)		
Divorced/Widowed	14 (1.4)		
Medical history			
Diabetes [¥]	37 (5.2)		
High blood pressure [¥]	58 (8.9)		
Heart disease [*]	9 (1.4)		

Table 4.3: Socio-demographic characteristics (N=858)

4.2.2 Anthropometric, body fat percentage and clinical measurements

Almost 60% of the participants in this study were overweight and obese (**Table 4.4**). The majority of the males had significantly lower percentage of body fat compared to females (p<0.001). Males had larger waist circumference (p=0.017) and higher systolic blood pressure than females (p=0.012). However, there was no significant difference between abnormal waist circumference and Parathyroid Hormone (PTH) among sex.

	Total	Male	Female	<i>p</i> -value
	(n= 858)	(n=77)	(n=781)	(GLM)
	n (%)	n (%)	n (%)	
BMI groups $(kg/m^2)^{\text{f}}$				
Underweight (<17.5)	32 (4.1)	2 (2.0)	30 (4.3)	0.805
Normal (17.5-22.9)	316 (36.4)	33 (40.4)	283 (36.0)	
Overweight (23.0- 27.9)	207 (24.2)	15 (23.3)	192 (24.3)	
Obese (≥28.0)	303 (35.3)	27 (34.3)	276 (35.4)	
Body Fat Tertile (BF %)				
Tertile 1 (≤24.77)	183 (33.3)	31 (82.0)	152 (29.5)	< 0.001
Tertile 2 (24.78- 31.62)	184 (31.3)	5 (9.7)	179 (33.0)	
Tertile 3 (≥31.63)	183 (35.4)	3 (8.3)	180 (37.5)	
Body fat (BF %)	28.1 ± 8.0	20.0 ± 7.2	28.7 ± 7.8	0.001
PTH (pmol/L)	6.1 ± 3.7	6.1 ± 5.9	6.1 ± 3.5	0.930
Waist circumference	80.4 ± 11.7	88.15 ± 14.5	79.6 ± 11.1	0.017
Abnormal waist	398 (46.5)	27 (35.2)	371 (46.5)	0.182
circumference (cm)	C ^			
Systolic BP (mmHg)	122.9 ± 19.2	130.4 ± 12.1	122.1 ± 19.6	0.012
Diastolic BP (mmHg)	77.9 ± 13.0	79.7 ± 10.1	77.8 ± 13.2	0.916
Physical Activity				
Low	282 (41.8)	18 (31.1)	264 (42.8)	0.171
Moderate	128 (19.2)	12 (22.7)	116 (18.8)	
High	290 (39.1)	25 (46.3)	265 (38.4)	
Sun Exposure Score	0.26 ± 0.62	0.19 ± 0.15	0.26 ± 0.64	0 725
Sun Exposure Score	0.20 ± 0.02	0.17 ± 0.13	0.20 ± 0.04	0.723
Sun Avoidance Score	3.50 ± 1.18	2.33 ± 1.30	3.61 ± 1.11	< 0.001

Table 4.4: Anthropometric, body fat percentage, Parathyroid Hormone(PTH), physical activity and sun exposure avoidance score by sex

The data represent average \pm SD, number (%)

[£] Classification according to World Health Organization (2004) for Asian Country

4.2.3 Physical activity and sun exposure avoidance score

There was an approximately equal proportion of participants with low level and high level of physical activity (**Table 4.4**). More males had high level of physical activity (46.3%) compared to females. Females had a higher average score of sun avoidance than males (p<0.001) (**Table 4.4**). The percentage of females wearing veils, cap/ hat, long sleeves, gloves, long pants or skirts, wearing umbrella and use of skin lotion were higher than males (**Figure 4.2**). This might explain the significance of higher score of sun avoidance among females compared to males. Malay participants had the highest average score of sun avoidance compared to Chinese, Indian and others (results not shown).



Figure 4.2: Comparison of sun avoidance behaviour and clothing styles

4.2.4 Prevalence of vitamin D deficiency

Following the US Endocrine Society Clinical Practice Guideline's definition (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney et al., 2011), vitamin D deficiency (<20 ng/ml) was diagnosed in 579 (67.4%) participants. Among those with vitamin D deficiency, Indians showed the highest proportion of vitamin D deficiency compared to Malays, others and Chinese (**Figure 4.3**).



Figure 4.3: Prevalence (%) of vitamin D deficiency among ethnicity

4.3 Association of vitamin D and its risk factors- Univariate analysis

4.3.1 Socio-demographic characteristics

In **Table 4.5**, participants less than 30 years of age had the lowest mean serum 25(OH)D level compared to the participants with ages 30 years and above. The mean for the serum 25(OH)D level increased with age (p= 0.001). Females had lower serum 25(OH)D level compared to males (p<0.001). Indians had the lowest mean serum 25(OH)D level compared to Malay, Chinese and others (p<0.001). There was no significant difference between those with diabetes, high blood pressure and heart disease with serum 25(OH)D level (**Table 4.5**).

	Serum 25(OH)D	
Characteristics	Weighted mean, \overline{x} (95% CI)	<i>p</i> -value
Age (years)		
<30	16.76 (15.56 to 17.97)	0.001
30-39	17.24 (16.36 to 18.11)	
40-49	17.81 (16.86 to 18.78)	
≥ 50	20.69 (19.11 to 22.27)	
Sex		
Male	25.27 (23.41 to 27.14)	<0.001
Female	17.24 (16.68 to 17.80)	
Ethnicity		
Malay	16.57 (16.06 to 17.09)	< 0.001
Chinese	25.44 (23.84 to 27.05)	
Indian	15.47 (13.98 to 16.96)	
Others	19.55 (14.18 to 24.92)	
BMI (kg/m^2)		
Underweight (<17.5)	17.29 (14.15 to 20.43)	< 0.001
Normal (17.5-22.9)	19.20 (18.23 to 20.17)	
Overweight(23.0- 27.9)	18.30 (17.02 to 19.57)	
Obese (≥28.0)	16.56 (15.82 to 17.30)	
Body Fat Tertile %		
Tertile 1 (≤24.77)	18.92 (17.58 to 20.27)	0.037
Tertile 2 (24.78- 31.62)	16.93 (15.83 to 18.02)	
Tertile 3 (≥31.63)	16.90 (15.92 to 17.88)	
Waist Circumferences (cm)		
Male		
<90	26.06 (23.54 to 28.57)	0.227
≥90	23.83 (21.20 to 26.46)	
Female	$17.90(17.01 \pm 19.50)$	0.022
<80	1/.80(1/.01 to 18.59)	0.023
$\geq \! 80$	10.51 (15.74 to 17.29)	
Medical History		
Diabetes (N=708)		
No	18 25 (17 51 to 18 99)	0.537
Ves	17.51 (15.31 to 19.72)	0.007
High blood pressure (N-708)	(10.01 (10.01 (0 17.72)	
No	18.17 (17.42 to 18.91)	0.703
Ves	18.65 (16.29 to 21.01)	0.705
Heart disease (N-600)	10.05 (10.27 to 21.01)	
No	18.41.(17.62 to 10.20)	0.507
Vec	17.52 (15.02 to 17.20)	0.307
103	17.52 (15.05 to 20.02)	

Table	4.5:	Weighted	mean	serum	25(OH)D	of	socio-demographic
charac	teristi	es, anthropo	ometric	measure	ments and l	oody	fat percentage

4.3.2 Anthropometric measurements and body fat percentage

Table 4.5 shows that obese participants had the lowest mean serum 25(OH)D level compared to underweight, overweight and normal weight participants (p<0.001). Participants with a higher composition of body fat percentage had a lower serum 25(OH)D level (p= 0.037). Females with larger waist circumference showed a lower mean serum 25(OH)D (p= 0.023). Adiposity was found to be negatively associated with serum 25(OH)D level; BMI (β = -0.23, p<0.001) and fat percentage (β = -0.14, p<0.005) (**Table 4.6**).

4.3.3 Parathyroid Hormone (PTH), blood glucose, blood pressure, sun exposure avoidance score and physical activity

There was a significant association between serum 25(OH)D level and Parathyroid Hormone (PTH) (p<0.001) (**Table 4.6**). **Table 4.6** also shows that there were negative and positive associations between serum 25(OH)D level with the sun avoidance score (p<0.001) and systolic blood pressure (p<0.005) respectively. No significance was found in blood glucose, sun exposure score and physical activity (p>0.005) with serum 25(OH)D level.

	Serum 25(OH)D	
	Unstandardized $\beta \pm SE$	<i>p</i> -value
Age	0.13 ± 0.03	< 0.001
Sex	-3.91 ± 0.38	< 0.001
Ethnicity		
Chinese	Reference	
Malay	-8.87 ± 0.86	< 0.001
Indian	-9.98 ± 1.12	< 0.001
Others	-5.90 ± 2.85	0.039
Body Mass Index (BMI) (kg/m ²)	-0.23 ± 0.05	< 0.001
Fat percentage (%)	-0.14 ± 0.04	0.002
Waist circumference (cm)	-0.32 ± 0.02	0.152
PTH (pmol/L)	-0.44 ± 0.09	< 0.001
Blood glucose (mmol/L)	0.12 ± 0.19	0.519
Physical Activity (METs/minute)	1.17 ± 0.33	0.929
Sun Avoidance Score	-1.03 ± 0.27	< 0.001
Sun Exposure Score	0.03 ± 0.53	0.955
Systolic Blood Pressure	0.03 ± 0.02	0.029
Diastolic Blood Pressure	0.02 ± 0.02	0.334

Table 4.6.	Association of	vitamin D k	aval and ite	rick factors
I ADIC 4.0.	Association of	vitamii D R	ever allu its	115K 14C1015

Sex: Males= 1, Females= 2

4.4 Association of vitamin D and its risk factors- Multivariate analysis

Females, ethnicity (Malays and Indians) (p<0.001), higher BMI and larger waist circumference (p<0.05) were significantly associated with lower serum 25(OH)D level in Model 2 and Model 3 respectively (**Table 4.7**). When the various adiposity indicators were included into the models separately, only BMI and waist circumference showed a significant association with serum 25(OH)D level (p<0.05), compared to body fat percentage (p>0.05). However, there were not much difference in R^2 values between Model 1 (Reference Model) and Models 2, 3, 4 (adjusted for adiposity indicators). Adiposity explained only less than 1% of the variability of serum 25(OH)D level.

	Unstandardized B				
	Model 1	Model 2	Model 3	Model 4	
Adiposity indicators					
BMI		-0.140*			
Waist circumference			-0.052*		
Fat percentage				-0.016	
Age	0.022	0.033	0.030	0.005	
Sex	-7.485**	-7.461**	-7.918**	-7.426**	
Ethnicity					
Chinese	Reference	Reference	Reference	Reference	
Malays	-9.030**	-8.512**	-8.763**	-9.046**	
Indians	-9.550**	-9.070**	-9.138**	-9.241**	
Others	-5.473	-4.965	-5.289	-2.845	
Sun Avoidance Score	-0.047	-0.081	-0.055	0.413	
Systolic Blood Pressure	0.001	0.013	0.011	0.011	
R^2	32.0%	32.8%	32.6%	32.3%	

Table 4.7: Different adiposity indicators affecting vitamin D level

#PTH was excluded in the analysis as it is an outcome of serum 25(OH)D level

** *p*<0.001, **p*<0.05

Sex: Males= 1, Females= 2

Model 1 = Controlled for age, sex, ethnicity, sun avoidance score, systolic blood pressure

Model 2 = Model 1 + BMI

Model 3= Model 1+ Waist Circumference

Model 4 = Model 1 + Fat Percentage

4.5 Contribution of risk factors on vitamin D level

The difference in R^2 values showed that ethnicity (R^2 = 16.9%) was the biggest contributor in affecting the level of serum 25(OH)D, followed by sex (R^2 = 7.1%) and adiposity (BMI) (R^2 = 0.8%) (**Table 4.8**).

	Unstandardized B			
_	Model 1 (Reference Model)	Model 2	Model 3	
BMI	-0.140*	-0.145*	-0.327**	
Age	0.033	0.022	0.138**	
Sun Avoidance Score	-0.081	-0.638*	-0.377	
Systolic Blood Pressure	0.013	0.025	0.021	
Ethnicity				
Chinese	Reference	Reference		
Malays	-8.512**	-8.317**		
Indians	-9.070**	-9.558**		
Others	-4.965	-5.913*		
Sex	-7.461**		-7.269**	
R^2	32.8%	25.7%	15.9%	
Difference in R^2		7.1%	16.9%	

** *p*<0.001, **p*<0.05 Sex: Males= 1, Females= 2

Model 1= Predictors: age, sun avoidance score, systolic blood pressure, BMI, ethnicity, sex Model 2= Model 1 - Sex

Model 3= Model 1 - Ethnicity

Difference in $R^2 = R^2$ in Model 1 (Reference Model) - R^2 in Model 2/ Model 3

CHAPTER 5: DISCUSSION

5.1 **Response rate, power of sample and sample weight**

Response rate is one of the important factors that affect the generalizability of the results, as it determines how well the sample represents a population. This study showed that the response rate of school was 75% which was quite high, while the response rate of teachers was 41%. The reasons of the non-response might be teachers unable to leave the classroom during teaching hours or they were busy with examination or attending other external courses. However, weightage was applied to correct the unequal selection probabilities and non-response. The sample size was acceptable as the power of our study was 100%. The response rate of returned questionnaires for all participants was also more than 80%. A good response rate of returned questionnaire would provide more valid results.

5.2 Descriptive characteristics

5.2.1 Socio-demographic characteristics

The age group of the participants were between 23 to 59 years. There were more participants aged 40 years and above similarly with the study by Rahman et al. (2004) and Moy et al. (2011). Middle-aged populations are at a higher risk to develop diseases without proper diet, physical activity and lifestyle control. The majority of our participants were females, mainly because the total average of male teachers only contributed to 20% out of the total teacher's population (Ministry of Education, 2011).

In addition, the majority of our participants were Malays which was similar with the data on the Malaysia Demographic Profile 2014. The majority of the Malaysian population are Malays (50.1%), followed by Chinese (22.6%), Indians (6.7%) and others (indigenous, Bumiputera Sabah Sarawak). However, there was a slightly less proportion of Chinese participants compared to the Malaysian population. The distribution of ethnic groups among teachers in government schools do not represent the general population in Malaysia.

Based on the medical history, very few of our participants reported with diabetes, high blood pressure and heart disease as they were relatively young (age ranged from 23 to 59 years old). These chronic diseases usually occurred among older adults (Chan, Teh, Lim, Lim, Yeo, Kee et al., 2015).

5.2.2 Anthropometric, body fat percentage and clinical measurements

5.2.2.1 Anthropometric measurements

a) Body Mass Index (BMI) (kg/m²)

The prevalence of obesity and overweight were high in our study. Findings by the National Health and Morbidity Survey IV (NHMS IV) (2011) showed that the prevalence of obesity increase from 4.4% in 1996 to 14.0% in 2006 and then increased more gradually to 15.1% in 2011. An estimation of 2.5 million of Malaysian adults were categorized as obese.

A similar finding was reported by Wan Mohamud et al. (2011) among 4428 adults (>18 years old) from five different selected regions in the East and Peninsular Malaysia. The prevalence of both overweight and obesity was more than 50% of the total population in which the prevalence of overweight and obesity were 33.6% (95% CI= 32.2, 35.0) and 19.5% (95% CI= 18.3, 20.7) respectively (Wan Mohamud, Musa, Md Khir, Ismail, Ismail, Kadir et al., 2011).

In our study, the prevalence of obesity among females was slightly higher than males. The NHMS IV (2011) study reported that out of 17,000 females surveyed, 29.4% of them were overweight while 15% were obese. This included the fact that the Malaysian women had the highest body mass index (BMI) in the South- East Asian region. Similarly, with the study reported by Wan Mohamud et al. (2011), it showed that there were more females who were obese 22.5% (95% CI= 20.9, 24.0) compared to males 14.1% (95% CI= 12.3, 15.9).
Malaysia has experienced a rapid economic development in recent decades, leading to the increase in urbanization and changes in lifestyle and nutritional status among the Malaysian population (Misra & Khurana, 2008). Urban dwellers tend to stay away from home while households tend to eat at the restaurants or fast food outlets for time saving. This actually changes the pattern of lifestyle and dietary intake.

On the other hand, many individuals adapted a more sedentary lifestyle and inactive behaviour as more time was spent doing indoor activities (Lau, Chong, Poh, & Ismail, 2013). The levels of physical activities related to work, household chores and transportations have declined dramatically and sedentary behaviours such as television viewing and internet or computer usage have increased substantially, leading to the reduction in physical activity levels (Brownson, Boehmer, & Luke, 2005). There might be the reasons of the increase on the prevalence of overweight and obesity among Malaysian adults.

b) Waist circumference

Waist circumference remains a simple and valid marker of abdominal and visceral fat. It provides a highly feasible and inexpensive method to monitor body fat distribution and identify individuals at greater risks of diseases in various settings (Beechy, Galpern, Petrone, & Das, 2012). In our study, males showed a significantly higher waist circumference compared to females. The main reason was due to the physiological characteristics of males.

The recommended cut-off waist circumference for Asians (90 cm in males and 80 cm in females) was used in our study, similar with the previous researchers (Moy & Bulgiba, 2011). There was no difference in the proportion of abnormal waist circumference between males and females, indicating that both sex were at risk of abdominal obesity. Abdominal obesity is closely related to the risk of high blood pressure, type 2 diabetes, insulin resistance and high blood pressure (Dunford & Doyle, 2011).

5.2.2.2 Body fat percentage (BF %)

Body fat percentage (BF %) was measured by using foot-to-foot Bioelectrical Impedance Analyzer (BIA). Our results showed that there was a significant difference in BF % between sex. Females had higher BF % than males. Our results supported the findings by Deurenberg et al. (2002) and Sim et al. (2014).

By nature, a female's body is developed to protect them and their potential foetus. As a result, females have more enzymes for storing fat and fewer enzymes for burning fat. Additionally, the hormone estrogen in females have activates fat storing enzymes and causes them to multiply. Biologically, this might be the main reason why females have a higher body fat percentage than males. Body fat percentage has been considered the best estimate of the future risk of cardiovascular disease (CVD).

5.2.2.3 Clinical measurements

a) Blood pressure

Blood pressure control is important in reducing the risk of cardiovascular disease among individuals, especially those with hypertension (Wilkins, Campbell, Joffres, Mcalister, Nichol, Quach et al., 2010). The findings from this study showed that there was a significant difference in mean systolic blood pressure between sex. Systolic blood pressure in females was reported to be slightly lower than males in the previous studies (Lim, Ding, Goh, Zaki, Suleiman, Maimunah et al., 2000; Moy & Bulgiba, 2011) which was similar with our findings.

A study among Americans also found that white and black males reported to have higher systolic blood pressure than white and black females (Sandberg & Ji, 2012). As males had higher blood pressure, they were at a higher risk of death due to hypertension-associated cardiovascular disease and coronary events, as reported in the Framingham study (Pencina, D'agostino, Larson, Massaro, & Vasan, 2009).

b) Parathyroid Hormone (PTH)

In our study, there was no significant difference between parathyroid hormone (PTH) among sex. PTH concentrations varied with age but were similar in both sex (Reis, Von Mühlen, & Miller, 2008). This supports our finding. PTH is a hormone in the body which functions to stabilize calcium metabolism. The release of PTH stimulates the reabsorption of calcium in the kidney, the resorption of calcium from the skeleton and enhances the production of 1,25-dihydroxyvitamin D (1,25(OH)₂D), the physiologically active molecule of vitamin D (Saquib, Von Mühlen, Garland, & Barrett-Connor, 2006). A reduction in PTH might lead to osteoporosis. Hypoparathyroidism has many life altering side effects such as muscle cramps and spasms, weakened of bones and teeth, seizures, decrease in energy and impaired nerve function.

5.2.3 Physical activity and sun exposure avoidance score

5.2.3.1 Physical activity

The majority our participants had a low level of physical activity. Malaysia has been recognized as having the potential to experience an increased level of chronic illnesses due to the high level of physical inactivity (Omar, Patterson, & Pegg, 2011). It is possible that the low level of physical activity were due to the use of technology and increased automation, besides more time being spent in sedentary work related activities or occupations including inactive commuting (Hu, Pekkarinen, Hänninen, Tian, & Jin, 2002; Chan, Spangler, Valcour, & Tudor-Locke, 2003).

Sex plays an important role in the participation of physical activity. Our study showed that more males had high level of physical activity compared to females. The nationwide Third National Health and Morbidity Survey (2006) reported that more than half of Malaysian adults were leading a sedentary lifestyle while accompanying physical inactivity in which females (65.1%) were found to be more inactive compared to males (55.4%). On the other hand, many other studies also reported similar findings (Pan, Cameron, Desmeules, Morrison, Craig, & Jiang, 2009; Poh, Safiah, Tahir, Haslinda, Norazlin, Norimah et al., 2010; Chu & Moy, 2013).

Based on the pattern of physical activity, males were physically more active and performed a relatively wider range of activities in each domain compared to females. In general, females experience more barriers in performing physical activity compared to males. Work, household chores and family care can cause females to have the lack of time to perform any physical activity that will eventually reach inadequate level of physical activity. A study by Nang et al. (2011) showed that Singaporean females had higher participation rate in household activity than males. They are generally more occupied in household and domestic domains such as child care, cooking, sweeping, vacuuming and scrubbing floor (Farahani, Tahmasbi, Asheri, Ashraf, Nedjat, & Kordi, 2011).

A similar trend was observed from other countries. In Saudi Arabia, a cross sectional study on prevalence of physical activity among Saudi adults aged 30 to 70 years proved that there were significantly more sedentary females (98.1%) than males (93.9%) (Al-Nozha, Al-Hazzaa, Arafah, Al-Khadra, Al-Mazrou, Al-Maatouq et al., 2007). This is similar with the study in Brazil among 1407 males and 1807 females which found that males were more active than females (Azevedo, Araújo, Reichert, Siqueira, Da Silva, & Hallal, 2007). A study that attempted to illustrate gender differences in physical activity showed that females encountered a lot of barriers than males such as personal safety, multiple roles and lack of time, which eventually limited their physical activity performance (Eyler, 2003). Ethnic, cultural and religious beliefs also influenced the performance of physical activity among females (Nakamura, 2002; Ransdell, Dinger, Huberty, & Miller, 2009).

Regular physical activity is an important part of a healthy lifestyle. Considerable evidence shows that a regular physical activity has many physical and mental health benefits, such as reduction in all-cause mortality and prevention of cardiovascular diseases, type II diabetes, hypertension, several types of cancers, osteoporosis, anxiety, and depression (Hardman & Stensel, 2009). Physical inactivity is an important factor associated with the risk of metabolic syndrome (Méndez-Hernández, Flores, Siani, Lamure, Dosamantes-Carrasco, Halley-Castillo et al., 2009; Chu & Moy, 2013).

5.2.3.2 Sun exposure avoidance score

Malaysia is a tropical country which has abundant sunshine, consistent temperature, high humidity and plentiful rainfall. In our study, the result showed that there was no significant difference between sex and sun exposure score (p>0.005). This could be due to our male and female participants worked indoor, as they were doing the same job. However, some of our participants might underestimate the number of days they might be exposed to sunlight.

Based on the previous studies on the level of physical activity as mentioned in Section 5.2.3.1, males were more active in doing physical activity compared to females. The chances of having more sunlight exposure among males might be higher compared to females.

Sun avoidance behaviour includes wearing veils, long sleeves, short sleeves, long pants/ skirts, short pants or skirts, wearing gloves, caps or hats, using lotion or cream sun protection and wearing umbrella. Our study supported the previous finding by Moy (2011) in which the sun avoidance score among females was higher than males (p<0.001). The main reason might be due to the religious requirement, especially among Malay females who were Muslims, in which they need to conceal most of their body parts while going outdoor.

Apart from that, females were more concerned about their skin complexion when going outdoor, since they tend to use sun screen lotion or using umbrella compared to males. Besides that, females, especially at the younger age, perceived fairer skin as a symbol of attractiveness and a measure of high social class. As a result, they avoided being exposed under sunlight (Ho-Pham, Nguyen, Lai, Eisman, & Nguyen, 2011; Potente, Coppa, Williams, & Engels, 2011).

5.2.4 Prevalence of vitamin D deficiency

Based on the Ministry of Science Technology and Innovation (MOSTI) reported in 2011, Malaysia received at least six hours of sunshine daily. However, many studies reported a high prevalence of vitamin D deficiency among Malaysian population. More than 50% of the participants had vitamin D deficiency (<20 ng/ml).

A study among postmenopausal Malaysian women showed that 27% and 71% of Malay women; 88% and 12% of Chinese women had hypovitaminosis D (20-40 ng/ml) and vitamin D insufficiency (10-20 ng/ml) respectively (Rahman, Chee, Yassin, & Chan, 2004). A study among women of child-bearing age living in Jakarta and Kuala Lumpur reported that 60% of women suffered from vitamin D deficiency (<20 ng/ml) (Green, Skeaf, Rockell, B.J., Lambert, Todd et al., 2008). Another study among Malay adults in Kuala Lumpur reported that 70% of them suffered from vitamin D deficiency (<20 ng/ml) (Moy & Bulgiba, 2011). In 2013, a study among urban and rural women in Kuala Lumpur and Palong, Negeri Sembilan also showed that the majority of urban women (43.9%) were vitamin D deficient (<12 ng/ml) compared to rural women (Nurbazlin, Chee, Rokiah, Tan, Chew, Nusaibah et al., 2013). A study among Malaysian men by Chin et al. (2014) in the Klang valley reported that vitamin D deficiency (<12 ng/ml) was uncommon among them, but still there was a significant proportion who had vitamin D insufficiency (12-20 ng/ml). Regardless of the differences in the cut-off definition of vitamin D deficiency used by Malaysian researchers, it was obvious that vitamin D deficiency among Malaysian population was severe.

Similar findings were reported by other countries rich with sunlight exposure (Mishal, 2001; Arya, Bhambri, Godbole, & Mithal, 2004; Hashemipour, Larijani, Adibi, Javadi, Sedaghat, Pajouhi et al., 2004; Atli, Gullu, Uysal, & Erdogan, 2005; Vupputuri, Goswami, & Gupta, 2006; Zargar, Ahmad, & Masoodi, 2007; Chailurkit, Aekplakorn, & Ongphiphadhanakul, 2011). A study among 1210 men and women in Iran between 20 to 69 years showed that the mean serum 25(OH)D level was only 8.24 ng/ml, showing that the prevalence of vitamin D deficiency was very high (Hashemipour, Larijani, Adibi, Javadi, Sedaghat, Pajouhi et al., 2004). In Sri Lanka (7°N), 40.5% of healthy females had vitamin D deficiency (20 ng/ml) (Islam, Akhtaruzzaman, & Lamberg-Allardt, 2006).

In North India (27°N), 96% of neonates (Sachan, Gupta, & Das, 2005), 78% of healthy hospital staff (Arya, Bhambri, Godbole, & Mithal, 2004) and 84% of pregnant women (Sachan, Gupta, & Das, 2005) were found to have vitamin D deficiency (<20 ng//ml). A study among 2641 adults aged 15 to 98 years in Bangkok, Thailand showed that 64.6% of them had vitamin D deficiency (<30 ng/ml) (Chailurkit, Aekplakorn, & Ongphiphadhanakul, 2011). High prevalence of vitamin D deficiency in South Asia may be explained by skin pigmentation and clothing styles.

This widespread of vitamin D deficiency had a deleterious effect on bone mineral homeostasis, which may lead to osteoporotic fracture (Arya, Bhambri, Godbole, & Mithal, 2004). Its deficiency not only increased the risk of osteoporosis, but also contributed to cardiovascular diseases, diabetes and certain types of cancers. In a meta-analysis carried out by Parkera et al. (2010), individuals with the highest level of serum 25(OH)D were associated with a 43% reduction in cardio-metabolic disorders (OR: 0.57; 95% CI: 0.48, 0.68).

5.3 Association of vitamin D and its risk factors

5.3.1 Socio-demographic characteristics

5.3.1.1 Age

A lower vitamin D level had been demonstrated to be more prevalent with advancing age in most studies (Dubbelman, Jonxis, Muslkiet, & Saleh, 1993; Jacques, Felson, Tucker, Mahnken, Wilson, Rosenberg et al., 1997). Aging decreases the skin's capacity to produce vitamin D (Maclaughlin & Holick, 1985). There is also a decrease in the hydroxylation of vitamin D and in the response towards intestinal mucosa to circulate vitamin D in elderly people (Heaney, 1999). Older individuals are especially at risk of developing vitamin D deficiency due to the decrease in the skin capacity to synthesize vitamin D, low sunshine exposure and low dietary vitamin D intake (Wicherts, Schoor, Boeke, Visser, Deeg, Smit et al., 2007).

Our findings contradicted the existing evidence in which older participants seemed to have higher serum 25(OH)D level compared with the younger participants. Similar results were found in studies among postmenopausal women in Malaysia (Rahman, Chee, Yassin, & Chan, 2004), urban population in Vietnam (Ho-Pham, Nguyen, Lai, Eisman, & Nguyen, 2011) and male population in the Klang valley (Chin, Ima-Nirwana, Ibrahim, Mohamed, & Wan Ngah, 2014) in which serum 25(OH)D level did not decrease with age. Instead, 25(OH)D level remained more or less the same from age 20 to 60 years old in Iranian men (Masoompour, Sadegholvaad, Larijani, & Ranjbar-Omrani, 2008). This could be because younger individuals had higher tendency to work indoor, besides perceiving that having a fairer skin is a symbol of high social class and attractiveness (Ho-Pham, Nguyen, Lai, Eisman, & Nguyen, 2011). As a result, they avoided being exposed to sunlight.

However, the exact reason for the positive association between serum 25(OH)D level and age was not clearly known. Age turned insignificant in the multivariate analysis after being adjusted for other cofounders. The main reason might be because age was also affected by other factors. In addition, our participants were in the range of ages from 23 to 59 years old in which they were relatively healthy, compared to the elderly. A further study should be carried out to explore the serum 25(OH)D level among adults over a wider spectrum of age.

5.3.1.2 Sex

Sex was significantly associated with serum 25(OH)D level in the univariate analysis. This remained statistically significant in the multivariate analysis after adjusting for all cofounders. In our study, the mean serum 25(OH)D level among females (17.24 ± 6.81 ng/ml) was significantly lower, compared to males (25.27 ± 7.80 ng/ml). The present study supported the previous findings (Lips, 2007; Chan, Chew, Vijay, Lim, Tan, Hazizi et al., 2009; Rahnavard, Eybpoosh, Homami, Meybodi, Azemati, Heshmat et al., 2010; Moy & Bulgiba, 2011), proving that females had a higher risk of vitamin D deficiency compared to males.

A possible reason contributing to this difference could be explained by their clothing style. This findings showed that there was significant negative correlation between serum 25(OH)D level and sun avoidance score. The sun

avoidance score among females was higher than males. Most females, especially the Muslims, covered up most of their bodies by wearing veils, long sleeves, long skirts/ pant, using umbrella and sun block lotion for avoiding sunlight exposure compared to males. This reduce the duration of exposure, which eventually reduced the production of levels of serum 25(OH)D in their body. Similar findings were also reported elsewhere (Mishal, 2001; Harinarayan, 2005; Lips, 2007; Moy, 2011).

On the other hand, there was a cultural perception among Asians, especially females, which prefers fairer skin (Li, Min, Belk, Kimura, & Bahl, 2008; Ho-Pham, Nguyen, Lai, Eisman, & Nguyen, 2011). As a result, they might avoid sunlight exposure, wearing clothes which cover most of their body parts and using umbrella or sun block lotion when going outdoor.

5.3.1.3 Ethnicity

In our study, ethnicity was significantly associated with serum 25(OH)D level in both univariate and multivariate analyses. A study by Rahman et al. (2004) showed that Malay women had lower serum 25(OH)D level (<10 ng/ml) compared to Chinese women. Nurbazlin et al. (2013) reported that a higher percentage of Indian and Malay women had lower vitamin D level in the range of 12-20 ng/ml, compared to Chinese. Meanwhile, Chin et al. (2014) also revealed a similar finding in which Malay men had lower vitamin D level (12-20 ng/ml) compared to Chinese men. Our study had similar findings as above.

Indians and Malays had a darker skin color compared to Chinese, where Indians, Malays and Chinese had the Fitzpatrick skin type VI; V and VI; III and IV respectively (Sng, Koh, Siong, & Choo, 2009). Higher melanin contents in dark skin can inhibit the vitamin D synthesis (Rahman, Chee, Yassin, & Chan, 2004). Tsiaras et al. (2011) reported that those with darker skin pigmentation or higher melanin content required longer sun exposure compared to those with lighter skin in order to produce a similar amount of vitamin D level needed in the body (Tsiaras & Weinstock, 2011).

5.3.1.4 Medical History

Medical history on diabetes, high blood pressure, heart disease and high cholesterol were enquired. There was no significant difference between diabetic individuals with serum 25(OH)D level. Our findings contradicted with the previous findings. Most of the studies showed that diabetics had lower serum 25(OH)D level (Scragg, Sowers, & Bell, 2004; Mattila, Knekt, Männistö, Rissanen, Laaksonen, Montonen et al., 2007; Lu, Yu, Pan, Hu, Franco, Li et al., 2009; Moy & Bulgiba, 2011). Besides that, high blood pressure and heart diseases were not associated with serum 25(OH)D level in our study. Our findings contradicted with the previous findings (Martins, Wolf, Pan, Zadshir, Tareen, Thadhani et al., 2007; Scragg, Sowers, & Bell, 2007; Moy & Bulgiba, 2011). The main reason might be due to the small number of our participants reported with diabetes, high blood pressure and heart disease. None of them had high cholesterol. These small sample sizes may not have adequate power to detect significant association with serum 25(OH)D level.

5.3.2 Anthropometric measurements and body fat percentage

Obesity is a well-established risk factor for cardiovascular disease (Rimm, Stampfer, Giovannucci, Ascherio, Spiegelman, Colditz et al., 1995; National Institute of Health & Lung and Blood Institute., 1998; World Health Organization, 2005) which leads to the increase in mortality and morbidity in both developed and developing countries (World Health Organization, 2000). It is recognized as a major determinant of other non-communicable diseases such as respiratory problems, gallbladder diseases, cancers and musculoskeletal disorders (Danaei, Ding, Mozaffarian, Taylor, Rehm, Murray et al., 2009). Obese individuals will be more susceptible to cardiovascular diseases (Baz-Hecht & Goldfine, 2010).

Obesity was also found to be negatively associated with serum 25(OH)D level. In many cross-sectional studies, the hypothesis made by several groups reported that serum 25(OH)D level were lower in obese individuals (BMI ≥ 30 kg/m²) (Snijder, Van Dam, Visser, Deeg, Dekker, Bouter et al., 2005; Cheng, Massaro, Fox, Larson, Keyes, Mccabe et al., 2010; Moy & Bulgiba, 2011; Chin, Ima-Nirwana, Ibrahim, Mohamed, & Wan Ngah, 2014).

5.3.2.1 Anthropometric measurements

Two anthropometric measurements, i.e. Body Mass Index (BMI) and waist circumference were used. Serum 25(OH)D level was significantly associated with BMI in both univariate and multivariate analyses. Lower serum 25(OH)D level was significantly associated with higher BMI (Young, Engelman, Langefeld, Hairston, Haffner, Bryer-Ash et al., 2009). Similar results were reported in other studies (Liu, Song, Ford, Manson, Buring, & Ridker, 2005; Mcgill, Stewart, Lithander, Strik, & Poppitt, 2008; Rueda, Fernández-Fernández, Romero, De Osaba, & Vidal, 2008; Mccarty, 2009; Chin, Ima-Nirwana, Ibrahim, Mohamed, & Wan Ngah, 2014).

Waist circumference is a better estimate of visceral fat or abdominal obesity. Therefore, it may be a better indicator of health risk than BMI and fat percentage (overall obesity) alone (Dobbelsteyn, Joffres, Maclean, & Flowerdew, 2001; Flegal, Shepherd, Looker, Graubard, Borrud, Ogden et al., 2009). In our study, a larger waist circumference was not significantly associated with a lower serum 25(OH)D level in the univariate analysis; however, it was significant in the multivariate analysis. The reason was most probably due to the fact that waist circumference may be affected by several other factors such as sex, affecting the serum 25(OH)D level.

A significant and negative relationship was also found between serum 25(OH)D level and waist circumference in some other studies (Mcgill, Stewart, Lithander, Strik, & Poppitt, 2008; Rueda, Fernández-Fernández, Romero, De Osaba, & Vidal, 2008; Lu, Yu, Pan, Hu, Franco, Li et al., 2009; Mccarty, 2009; Moy & Bulgiba, 2011; Ardawi, Sibiany, Bakhsh, Qari, & Maimani, 2012; 141

Abiaka, Delghandi, Kaur, & Al-Saleh, 2013; Jääskeläinen, Knekt, Marniemi, Sares-Jäske, Männistö, Heliövaara et al., 2013). However, Chin et al. (2014) did not find any significant association between serum 25(OH)D level and waist circumference.

5.3.2.2 Body fat percentage

Based on the literature reviews, not many studies used the direct measure of body fatness such as Bioelectrical Impedance Analyzer (BIA) and Dual-energy X-ray absorptiometry (DXA). Most studies had largely been limited to BMI (Arunabh, Pollack, Yeh, & Aloia, 2003; Alemzadeh, Kichler, Babar, & Calhoun, 2008). In our study, we used the foot-to-foot BIA to measure adiposity instead of using the anthropometric measurements alone.

Our study found that a higher body fat percentage was significantly associated with lower serum 25(OH)D level in the univariate analysis, but not in the multivariate analysis; similar with the findings reported by Looker (2005), Nurbazlin et al. (2013) and Chin et al. (2014) . However, some studies found body fat percentage to be negatively associated with serum 25(OH)D level (Arunabh, Pollack, Yeh, & Aloia, 2003; Rahman, Chee, Yassin, & Chan, 2004).

Our early hypothesis supported that BIA is a better indicator of adiposity measurement since it is a direct measure of body fatness compared to the anthropometric measurements such as BMI and waist circumference. However, we did not get a significant result may be due to the reason that the foot-to-foot BIA machine used may not be as accurate as the direct segmental multi-142 frequency (DSM)-BIA. On the other hand, our participants also might not adhere to certain conditions to be followed such as fasting or avoid eating or drinking or avoid exercising before the measurement took place.

There were controversial results on the association of body fat percentage and adiposity indicators (BMI and waist circumference) with serum 25(OH)D level. The studies conducted by Arunabh et al. (2003) and Snijder et al. (2005) found that serum 25(OH)D level were more strongly associated with body fat percentage compared to BMI, indicating that it was adiposity, not simply body mass that influenced the serum 25(OH)D level. More studies should be carried out to study the effect of body fat percentage on serum 25(OH)D level in the future.

5.3.2.3 Summary: serum 25(OH)D level and adiposity

The main reason for the association between serum 25(OH)D level and adiposity may be explained by the characteristics of vitamin D itself as a fat soluble vitamin. Higher body fat is expected to reduce the availability of circulating vitamin D (Rahman, Chee, Yassin, & Chan, 2004), due to its characteristic as a fat soluble vitamin. Another explanation was proposed by Bell et al. (1985) in which vitamin D endocrine system in obese people was altered, with an increased production of 1,25-dihydroxyvitamin D exerting a negative feedback control on hepatic synthesis. Besides, obese individuals had higher fat content in their body that might probably block the vitamin D from being sequestered into the body that will eventually lower the circulation of serum 25(OH)D in the body. This will give rise to a reduced bioavailability of vitamin D metabolite which regulates the transcription of multiple gene products with pro-differentiative, anti-proliferative and immunomodulatory effects (Baz-Hecht & Goldfine, 2010).

Abdominal obesity had also been linked with the decrease in serum 25(OH)D level (Moy & Bulgiba, 2011; Ardawi, Sibiany, Bakhsh, Qari, & Maimani, 2012; Abiaka, Delghandi, Kaur, & Al-Saleh, 2013), increase in cardiovascular risk, development in hyperinsulinemia, insulin resistance, heart disease and high blood pressure (Sowers, 2003). Therefore, it is important to measure both the overall and abdominal or central obesity while accessing vitamin D related diseases in the future.

These three different measurements of adiposity indicators were comparable in explaining the variations contributed to the multivariate model after being adjusted for age, sex, ethnicity, systolic blood pressure and sun avoidance score. There might be other risk factors apart from adiposity indicators that contributed more on serum 25(OH)D level.

5.3.3 Parathyroid Hormone (PTH), blood glucose, blood pressure, sun exposure avoidance score and physical activity

5.3.3.1 Parathyroid Hormone (PTH)

Parathyroid hormone (PTH) is a major hormone maintaining normal serum concentrations of calcium and phosphate and is itself regulated through the levels of calcitriol and serum calcium. There were contradicting findings between PTH and serum 25(OH)D level. An inverse correlation have been reported between PTH and serum 25(OH)D level in some studies (Yan, Prentice, Zhang, Wang, Stirling, & Golden, 2000; Nakamura, Nashimoto, Matsuyama, & Yamamoto, 2001; Souberbielle, Cormier, Kindermans, Gao, Cantor, Forette et al., 2001) while others showed no significant association with serum 25(OH)D level (Green, Skeaff, Rockell, Venn, Lambert, Todd et al., 2008; Elsammak, Al-Wossaibi, Al-Howeish, & Alsaeed, 2011; Ho-Pham, Nguyen, Lai, Eisman, & Nguyen, 2011; Nurbazlin, Chee, Rokiah, Tan, Chew, Nusaibah et al., 2013).

A decrease in serum 25(OH)D level is generally associated with an increase in PTH, which is similar with our result in univariate analysis. There was an inverse correlation between serum 25(OH)D level and Parathyroid Hormone (PTH), as similarly reported in other studies (Souberbielle, Cormier, Kindermans, Gao, Cantor, Forette et al., 2001; Nakamura, 2002; Chin, Ima-Nirwana, Ibrahim, Mohamed, & Wan Ngah, 2014). However, PTH was excluded from the multivariate analysis as it is an outcome of serum 25(OH)D level. Vitamin D and PTH are both responsible for maintaining extracellular calcium homeostasis (Reis et al., 2007). Vitamin D increases the efficiency of intestinal calcium absorption whereas PTH is secreted in response to low circulating calcium concentrations. An elevated PTH secondary to low vitamin D will increase the calcium absorption from the skeleton at the expense of an increased risk of fracture. Secondary hyperparathyroidism may also increase the risk of hypertension, diabetes and osteoporosis (Paul, 2004).

Elevated PTH concentration is also known to be associated with cardiometabolic diseases (Zittermann, 2006; Lavie, Dinicolantonio, Milani, & O'keefe, 2013). A recent study published in 2011 followed a total of 2,312 patients over 14 years to investigate the association between serum parathyroid hormone (PTH) and vitamin D deficiency with cardiovascular risk (Kestenbaum, Katz, De Boer, Hoofnagle, Sarnak, Shlipak et al., 2011). They found that an elevated PTH was associated with 30% increase in the risk of heart failure while vitamin D deficiency was associated with 25% increase in the risk of getting heart attack and 9 to 29% increase for the risk of death.

As both elevated PTH and vitamin D deficiency are common among older populations, especially at the age 60 years old and above, it is important that adults get enough vitamin D and calcium to promote better health. Therefore, there is a need to monitor the levels of serum 25(OH)D, PTH and calcium.

5.3.3.2 Blood glucose

Vitamin D deficiency impairs insulin secretion of pancreatic β -cells and increases insulin resistance in target tissues, which play critical roles in type 2 diabetes development (Knekt, Laaksonen, Mattila, Härkänen, Marniemi, Heliövaara et al., 2008). There were contradicting findings on the association between blood glucose with serum 25(OH)D level. A decrease in blood glucose level is generally associated with low serum 25(OH)D level (Martins, Wolf, Pan, Zadshir, Tareen, Thadhani et al., 2007; Holick & Chen, 2008; Gagnon, Lu, Magliano, Dunstan, Shaw, Zimmet et al., 2011). However, our study did not show any significant association between blood glucose and serum 25(OH)D level, similarly as reported by Moy & Bulgiba (2011).

This might be due to our participants were relatively healthy. In addition, our small sample size of those with high level of blood glucose may not have adequate power to detect significant association within individual risk factors with serum 25(OH)D level.

5.3.3.3 Blood pressure

Serum 25(OH)D level was found to be significantly associated with systolic blood pressure in the univariate analysis, but not significant in the multivariate analysis. Our result contradicted with the previous findings (Moy & Bulgiba, 2011). However, a study among 27 158 participants in North Norway supported our findings (Jorde et al., 2010). There are many indications that vitamin D is involved in blood pressure regulation, the most important is 1,25-dihydroxyvitamin D which inhibits the renin mRNA expression (Li et al., 2002).

A small interventional trial conducted among vitamin D deficient elderly women demonstrated that 800 IU per day of oral vitamin D for 6 weeks lowered systolic blood pressure by 9.3% (Pfeifer et al., 2000). Besides that, a study by Forman et al. (2007) observed an independent inverse association between predicted 25(OH)D levels and risk of incident hypertension.

Meanwhile, diastolic blood pressure was not significantly associated with serum 25(OH)D level in both univariate and multivariate analyses. In the recent study reported by Franklin et al. (2009), it was suggested that cardiovascular risk among middle-aged and older people was often more accurately predicted by using systolic blood pressure measurements than diastolic blood pressure measurements. Systolic blood pressure is known to increase with age as a result of the hardening of the arteries while diastolic readings are particularly important in monitoring the blood pressure in younger individuals (Franklin et al., 2009).

5.3.3.4 Sun exposure avoidance score

The present study showed that sun exposure was not significantly associated with serum 25(OH)D level in both univariate and multivariate analyses. Our result contradicted the existing evidence (Moy & Bulgiba, 2011). Some participants may have underestimated the duration when they were exposed to sunlight. Future study should be carried out using photometer tool to measure the sunlight exposure more accurately, instead of using questionnaire alone.

The mean serum 25(OH)D level among Thais seemed to be higher compared to those reported in the West (Mckenna, 1992; Chapuy, Preziosi, Maamer, Arnaud, Galan, Hercberg et al., 1997). This might be due to the higher exposure to sunshine throughout the years since the latitude of Thailand is closer to the equator than the Western countries. A cohort study by Vieth et al. (2001) among young healthy Canadian women (age 18 to 35 years) in Toronto found that low level of serum 25(OH)D (<16 ng/ml) was reported during the winter month. Rucker et al. (2001) also reported a similar finding in Canadians of European ancestry.

Our results on sun avoidance score was similar with the previous finding (Moy, 2011) in which it was negatively associated with serum 25(OH)D level. At the same time, there was a significant difference in sun avoidance score between sex (p<0.001). The main significant reason might be because most of the females covered most of their body by wearing long sleeves, long skirts/pants, veils; and avoiding sunlight by using umbrella, as shown in Figure 4.2, compared to males.

Apart from that, studies in Turkey and Jordan (Alagöl, Shihadeh, Boztepe, Tanakol, Yarman, Azizlerli et al., 2000; Mishal, 2001) among females showed a strong relationship between serum 25(OH)D level and clothing. Serum 25(OH)D level decreased from females with Western clothing, going to traditional females with hijab and completely veiled females with *niqab*. In contrast, males in these countries had higher level of serum 25(OH)D compared to females.

By ethnicity, the sun avoidance score among Malays were higher compared to other ethnicity. This might be due to religious factors as the majority of Malays are Muslims. The majority of Muslims covered most of their body parts while going outdoor due to their religious belief. Therefore, this limited their skin exposure to sunlight for vitamin D synthesis. However, sun avoidance was not significantly associated with serum 25(OH)D level in the multivariate analysis after being adjusted for age, sex, ethnicity, BMI and systolic blood pressure.

5.3.3.5 Physical activity

Physical activity was not significantly associated with serum 25(OH)D level in both univariate and multivariate analyses. Our participants may have overestimated the time spent in conducting physical activities or the short form of International Physical Activity Questionnaire (IPAQ) may not be able to capture the physical activity information as accurate as a pedometer. However, there were studies that reported a significant association between physical activity with serum 25(OH)D level (Wat, Leung, Tam, & Kung, 2007; Ardawi, Sibiany, Bakhsh, Qari, & Maimani, 2012; Chin, Ima-Nirwana, Ibrahim, Mohamed, & Wan Ngah, 2014).

Generally, high level of physical activity is a surrogate measure of sun exposure. It was speculated that individuals who were physically active also tended to spend more time under the sun as sunlight is the main source of vitamin D (Houston, Cesari, Ferrucci, Cherubini, Maggio, Bartali et al., 2007). Previous studies had established that outdoor physical activity was more relevant to the vitamin D status of the population (Wat, Leung, Tam, & Kung, 2007; De Rui, Toffanello, Veronese, Zambon, Bolzetta, Sartori et al., 2014). However, the reason still remained unclear whether doing physical activity or exercise itself contributed to the elevated of serum 25(OH)D levels (Brock, Huang, Fraser, Ke, Tseng, Stolzenberg-Solomon et al., 2010).

Evidence from international epidemiological and research showed that those who participated in high levels of physical activity have less stores of body fat in their body (Dipietro, 1999). Since high BMI level was also associated with low serum 25(OH)D level, it can be suggested that obese individuals may tend to do less physical activity compared to those with a normal BMI level. Similarly, in the study by Al-Othman et al. (2012), it was reported that the lowest mean level of serum 25(OH)D was found in a physically inactive group $[17.7 \pm 1.6 \text{ nmol/l}]$ and 22.7 \pm 1.5 nmol/l] (*p*<0.05) (Al-Othman, Al-Musharaf, Al-Daghri, Krishnaswamy, Yusuf, Alkharfy et al., 2012).

5.4 Contribution of risk factors on vitamin D level

Among the significant factors associated with serum 25(OH)D level such as ethnicity, sex, BMI and waist circumference; we found that the strength of association for BMI and waist circumference was weak. The R^2 contributed to the model variation was only approximately 0.8%. The two most influential factors affecting serum 25(OH)D level were ethnicity and sex. Ethnicity as the proxy of skin pigmentation and sex based behaviors were more dominant in contributing to serum 25(OH)D level.

Located in the South East Asia region, Malaysia is among the most unique countries in the world. This uniqueness greatly is aided by its diversity of cultures. Malaysia consists of a multi-ethnic nation with a population of 30 million. The major ethnic groups are Malays which contributed almost half of the Malaysian population, followed by Chinese, Indians and others (Bumiputra Sabah and Sarawak). The difference in ethnicity among the Malaysian population resulted in the difference in the religious belief, skin pigmentation, clothing styles, lifestyle behaviour, physical activity patterns, dietary intake and socio-demographic characteristics within each ethnic group. Due to their different beliefs within each ethnicity and difference in lifestyle behaviors, this was the reason why some individuals covered their hair with veils, some wore long sleeves, some used lotion while going outdoors and some did physical activity regularly. All of these factors contributed to the different level of sunlight exposure among individuals, which eventually affected the level of serum 25(OH)D. In our study, Malays had the highest mean score in sun avoidance compared to other ethnicities. This might be due to their religious belief as the majority of them are Muslims that required them to cover most of their body parts or wearing hijab while going outdoors.

Besides that, skin pigmentation also affected the level of serum 25(OH)D. A darker skin colour may reduce the ability of the skin to synthesize vitamin D. Malays and Indians with darker skin colour had lower serum 25(OH)D level compared to Chinese. In summary, sex (sex based behaviours) and ethnicity (skin pigmentation) contributed more on serum 25(OH)D level compared to adiposity.

5.5 Limitations and strengths

There were some limitations in this study that warrant for discussion. Firstly, a cross-sectional study could not establish a causality between various risk factors and serum 25-hydroxyvitamin D (25(OH)D) level. Secondly, there were various methods to determine serum 25(OH)D level. A key problem was that some of the immunoassays underestimated 25(OH)D metabolites due to the differences in affinity between the antibodies or D-binding protein employed. Our method may differ from other studies.

The cut-off point to define vitamin D deficiency in our study may be different from others that were be based on functional or clinical outcomes, thus imposed difficulty in making comparison. The difference in the cut-off point to define vitamin D deficiency might affect the outcomes of the vitamin D status in different studies.

Besides that, we did not collect the data on dietary intake and vitamin D supplement used among participants. Recall bias, especially from IPAQ- short form and sun exposure avoidance questionnaires from our participants, may be another limitation in our study. Notwithstanding the above limitations, the present study had several strengths. Firstly, our sample size was reasonably large, which provided adequate power for the main analysis. Besides, a complex sample analysis was used in our study which required the weightage to be calculated in order to correct unequal selection probabilities and non-response. Our study also comprised of multi-ethnic adults, thus making it possible to infer to all adults in Kuala Lumpur.

On the other hand, we tested the association of serum 25(OH)D level with various adiposity indicators. To our knowledge, there was no other study in Malaysia investigating the contribution of various risk factors to serum 25(OH)D level. We established that sex (sex based behaviors towards sun exposure and avoidance) and ethnicity (skin pigmentation) were dominant in affecting serum 25(OH)D level, compared to other risk factors. Our findings have important implications for future studies.

CHAPTER 6: CONCLUSIONS & RECOMMENDATIONS

6.1 Conclusions

In conclusion, the prevalence of vitamin D deficiency (<20 ng/ml) among our participants was high (67.4%). In the univariate analysis; females, ethnicity, age and individuals with high BMI, large waist circumference, high body fat percentage, low level of Parathyroid Hormone (PTH), high systolic blood pressure and high avoidance towards sunlight exposure were at risk of vitamin D deficiency.

There were 24.2% and 35.3% of the participants who were overweight and obese respectively. Obesity is a major determinant of many non-communicable diseases such as cancers, musculoskeletal diseases, respiratory problem and can cause mortality. At the same time, the level of physical activity was low among this population.

In addition, we found that adiposity was significantly associated with serum 25(OH)D level. This was due to the characteristic of vitamin D itself as a fat soluble vitamin. Obese individuals tend to have higher adipose tissue in their bodies which eventually blocked the vitamin D from being sequestered and processed in the body. After adjusted for age, sex, ethnicity, systolic blood pressure and sun avoidance score; higher Body Mass Index (BMI) and larger waist circumference were inversely associated with lower serum 25(OH)D level. However, there was no significant association of body fat percentage with serum 25(OH)D level in multivariate model analysis. This contradicted with our

hypothesis in which BIA is a better indicator of adiposity measurement compared to anthropometric measurements.

In the multivariate analysis adjusting for age, sex, ethnicity, systolic blood pressure and sun avoidance score; ethnicity (Malays and Indians), females, higher Body Mass Index (BMI) and larger waist circumference remained significantly associated with lower serum 25(OH)D level.

Among the significant factors associated with serum 25(OH)D level; we found that the strength of association for BMI and waist circumference was weak. Adiposity (BMI) only contributed 0.8% to serum 25(OH)D level in the multivariate model. The two most influential factors affecting serum 25(OH)D level were ethnicity (16.9%) and sex (7.1%). Ethnicity as the proxy of skin pigmentation and sex based behaviors were more dominant in contributing to serum 25(OH)D level.

6.2 **Recommendations**

6.2.1 Future research

Due to the constraints faced by our study, we would like to suggest a cohort study to be carried out to establish the causality between various risk factors and serum 25(OH)D level. In addition, the study population should be recruited from a wider spectrum of age, more diverse occupation (indoor and outdoor with different exposure towards sunlight) and higher proportion of Chinese and Indians so that the results can be better inferred to all adults in Malaysia.

More objective measurements such as pedometer and photometer should be used in the future to measure the physical activity and sun exposure respectively in order to get more precise results, instead of using questionnaires alone. Although body fat percentage was not significantly associated with serum 25(OH)D level in our study, it should be included as one of the indicators used in future studies as it is a direct measure of adiposity, compared to anthropometric measurements. In addition, a direct segmental multi-frequency (DSM)-BIA should be used rather than the foot-to-foot BIA as it may not be as accurate as DSM-BIA. On the other hand, a further study should also be carried out to determine either indoor or outdoor physical activity itself contributes to serum 25(OH)D level.

6.2.2 Public Health Intervention

As the prevalence of vitamin D deficiency is high in our country, actions should be taken to address this problem. Health screening on vitamin D deficiency should be carried out routinely, especially among the high risk groups such as females and those from the Malay and Indian ethnicity. This is because there are no symptoms of vitamin D deficiency at the early stage. Health education should be given on how to prevent vitamin D deficiency. This health education should be targeted in gender based behaviors such as exposure under sunlight 20 to 30 minutes per day to get enough sunshine.

Since obesity is a risk for cardiovascular diseases and factor affecting the serum 25(OH)D level, there is an urgent need to carry out effective health promotion programs to reduce obesity. Individuals, especially those who are obese, should modify their lifestyle behaviors and dietary pattern intake. They should reduce the time spent doing sedentary activities such as watching television and sitting or using computer. They should be encouraged to be involved in sunshine-related activities such as walking in the morning and gardening.
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LIST OF PUBLICATIONS AND PAPERS PRESENTED

The following works have been accomplished during candidature:

Published paper:

Shafinaz I.S., & Moy, F.M. Vitamin D level and its association with adiposity among multi ethnic adults in Kuala Lumpur, Malaysia: A cross sectional study. *BMC Public Health*. Published online before print March 7, 2016.

Conference presentation (Poster):

Shafinaz, I.S, & Moy, F.M. (2014, June). Vitamin D status and its association with adiposity among multi ethnic teachers in Wilayah Persekutuan Kuala Lumpur. In Tee E.S., *Optimal Nutrition for Future Generation: Proceedings of the 30th Scientific Conference of the Nutrition Society of Malaysia, Kuala Lumpur.*