UNIVERSITI PUTRA MALAYSIA

RELATIONSHIPS BETWEEN DIETARY ACID LOAD, GENETIC FACTORS AND CARDIOMETABOLIC SYNDROME ON RISK OF OSTEOPOROSIS AMONG POSTMENOPAUSAL CHINESE WOMEN

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By

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Evidence is growing that high dietary acid load (DAL), and presence of cardiometabolic syndrome (CMS) might accelerate the rate of bone loss while gene polymorphisms are related to bone deterioration. However, data on DAL among Asian population was scarce with the exception of Hong Kong Chinese. At the same time, empirical evidence showed no clear consensus on the influence of DAL on bone health. The plausible mechanisms of high DAL on bone loss have remained understudied. The unexplored consideration between the links of DAL with CMS traits and single nucleotide polymorphism (SNP) on bone health may explain the contradictory findings. Thus, this study aimed to investigate the relationships between dietary acid load, genetic factors and cardiometabolic syndrome on risk of osteoporosis among postmenopausal Chinese women. At the same time, the study also sought to estimate DAL, vitamin D status, CMS traits and bone resorption rate among postmenopausal women, and to examine the relationships between sociodemographic background, lifestyle factors, anthropometry parameters and biochemical indices with risk of osteoporosis. Taking all together, DAL, CMS and genetic model on risk of osteoporosis among postmenopausal women was developed.

This was a cross-sectional study with a total of 211 eligible postmenopausal women were recruited from seven affiliates of the National Council of Senior Citizens Organizations Malaysia. Dietary intake of respondents was assessed using validated interviewer-administered semi-quantitative food frequency questionnaire (FFQ) while DAL was estimated using potential renal acid load (PRAL) and net endogenous acid production (NEAP). Blood was drawn for biochemical parameters and Agena® MassARRAY genotyping analysis was used to identify the IGF1 and IL6 genotypes. Serum collagen type 1 cross-linked C-telopeptide (CTX1) was used as the surrogate bone marker for rate of bone resorption. Interaction between DAL and genetic

polymorphisms as well as DAL and CMS were assessed using linear regressions and structural equation modelling.

Mean age of respondents was 66.7 ± 6.6 years and mean duration of menopause was 16.1 ± 7.8 years. Approximately 45% of the respondents had low-income and mean years of education was 8.0 ± 4.6. The means of weight and height were 57.9 ± 9.5 kg and 154.0 ± 4.02 cm, respectively. More than one-third of the respondents failed to achieve the recommended duration of physical activity while closed to half of them were poor sleepers. Approximately 70% of the respondents were either serum 25-hydroxyvitamin D deficient or inadequate. Mean PRAL score was 13.8 ± 19.1 mEq/day while mean NEAP score was 72.6 ± 29.3 mEq/day, and were relatively higher than other studies. The mean of CTX1 was 0.45 ± 0.2 µg/L. There were 55.9%, 47.9% and 67.3% of the respondents carried IL6 -572 CC genotype, IGF1 rs35767 CC genotype and IGF1 rs7136446 TT genotype, respectively. In IL6 -174 G/C, all respondents carried the GG genotype, with no C allele was found among the respondents.

Binary correlation analysis indicated that age ($r = -0.18$ $p<0.05$) and height ($r = 0.14$, $p<0.05$) were weakly but significantly correlated with bone resorption. Multiple linear regression showed that younger women, poorer sleeper, higher acidity diet consumption and being IL6 -572 CC genotype carrier were at higher risk of bone resorption. Structural equation modelling demonstrated similar findings with regression analysis, with the addition of the presence of CMS and low vitamin D serum level were the risk factors for higher bone resorption. Besides, there was an unexpected negative association between CMS traits and bone resorption. On the other hand, years of menopausal, years of education, number of diseases, physical activity, weight, BMI, waist circumference, IGF1, DAL-SNPs and DAL-CMS interaction effects were not associated with bone resorption.

In conclusion, it is generally acknowledged pathophysiology of osteoporosis and rate of bone loss is a complex process, with multiple factors are involved. Taken together, the current study provides new insight for scientific research on the risk factors for osteoporosis among postmenopausal Chinese women. It is of paramount important to delineate the possible correlations between dietary acid load, CMS traits and genetic factors with bone resorption in other studies. Future studies should also explore how other SNP candidates such as vitamin D receptor (VDR), estrogen receptor beta (ERβ) and transforming growth factor-β1 (TGF-β1) may correlate with risk of bone resorption among postmenopausal women.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

HUBUNGAN ANTARA ASID PEMAKANAN, FAKTOR GENETIK DAN SINDROM KARDIOMETABOLIK PADA RISIKO OSTEOPOROSIS DALAM KALANGAN WANITA MENOPAUS CINA

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Semakin banyak bukti yang menunjukkan bahawa kandungan asid pemakanan yang tinggi (DAL), dan kehadiran sindrom kardiometabolik (CMS) mungkin mempercepatkan kadar kehilangan tulang sementara gen polimorfisme berkait dengan kerosakan tulang. Walau bagaimanapun, data ke atas DAL dalam kalangan populasi sukar diperolehi kecuali populasi orang Cina di Hong Kong. Pada masa yang sama, bukti empirik menunjukkan konsensus jelas tentang pengaruh DAL ke atas kesihatan tulang. Mekanisme jelas DAL tinggi ke atas kehilangan tulang masih kurang dikaji. Pertimbangan di antara perkaitan DAL dengan ciri-ciri CMS dan polimorfisme nukleotid tunggal (SNP) ke atas kesihatan tulang yang kurang dikaji mungkin boleh memperjelaskan dapatan yang saling bercanggah. Oleh itu, kajian ini bertujuan untuk mengkaji perhubungan di antara beban asid pemakanan, faktor genetik dan sindrom kardiometabolik ke atas risiko osteoporosis dalam kalangan wanita Cina pada peringkat pasca-menopaus. Pada masa yang sama, kajian juga ingin menganggarkan DAL, status vitamin D, ciri-ciri CMS dan kadar penyerapan semula tulang dalam kalangan wanita pasca-menopaus, dan untuk mengkaji perhubungan di antara latar belakang sosio-demografi, faktor gaya hidup, parameter antropometri dan indeks bio-kimia dengan risiko osteoporosis. Secara keseluruhannya, DAL, CMS dan model genetik ke atas risiko osteoporosis dalam kalangan wanita pasca-menopaus telah dibangunkan.

Ini adalah satu kajian merentas bahagian dengan sejumlah 211 orang wanita pasca-menopaus yang layak direkrut dari tujuh agensi Majlis Organisasi Warga Emas Kebangsaan Malaysia. Pengambilan pemakanan responden dinilai menggunakan soal-selidik frekuensi makanan separa-kuantitatif yang dikendalikan oleh pengkaji (FFQ) sementara DAL dianggarkan menggunakan beban asid renal berpotensi (PRAL) dan pengeluaran asid endogenus (NEAP). Darah dambil untuk parameter bio-kimia dan

analisis genotaip Agena® MassARRAY digunakan untuk mengenalpasti IGF1 dan genotaip IL6. Kolagen serum jenis 1 telopeptid C-bersilang (CTX1) digunakan untuk menggantikan penanda tulang untuk kadar penyerapan semula tulang. Interaksi di antara DAL dan polimorfisme genetik begitu juga dengan DAL dan CMS dinilai menggunakan regresi linear dan pemodelan persamaan berstruktur.

Min umur responden adalah 66.7 ± 6.6 tahun dan min jangka masa menopaus 16.1 ± 7.8 tahun. Kira-kira 45% responden mempunyai pendapatan yang rendah dan min tahun pendidikan adalah 8.0 ± 4.6. Min berat dan tinggi masing-masing adalah 57.9 ± 9.5 kg dan 154.0 ± 4.02 cm. Lebih dari satu pertiga responden gagal mencapai jangka masa aktiviti fizikal yang disarankan sementara hampir separuh bilangan itu adalah mereka yang tidak dapat tidur dengan baik. Dianggarkan 70% subjek kurang serum 25-*hydroxyvitamin* D atau tidak mengambil serum tersebut secukupnya. Min skor PRAL adalah 13.8 ± 19.1 mEq/hari sementara min skor NEAP adalah 72.6 ± 29.3 mEq/hari, dan ia lebih tinggi dari kajian-kajian lain. Min CTX1 adalah 0.45 ± 0.2 µg/L. Terdapat 55.9%, 47.9% dan 67.3% responden yang membawa genotaip IL6 -572 CC, IGF1 rs35767 CC dan IGF1 rs7136446 TT, masing-masing. Dalam IL6 -174 G/C, semua subjek membawa genotaip GG, dengan tidak ada allele C ditemui dalam kalangan subjek.

Analisis korelasi binari menunjukkan bahawa usia ($r = -0.18$ $p<0.05$) dan ketinggian ($r = 0.14$, $p<0.05$) adalah signifikan tetapi lemah berkorelasi dengan penyerapan semula tulang. Regresi linear pelbagai menunjukkan bahawa wanita lebih muda, lebih kurang tidur, mengambil makanan yang tinggi kandungan asidnya dan pembawa genotaip IL6 -572 CC mempunyai risiko lebih tinggi dalam penyerapan semula tulang. Pemodelan persamaan berstruktur menunjukkan dapatan yang hampir sama dengan analisis regresi, dengan tambahan kehadiran CMS dan aras serum vitamin D yang rendah, adalah faktor-faktor risiko penyerapan semula tulang yang lebih tinggi. Di samping itu, terdapat perkaitan negatif di antara ciri-ciri CMS dan penyerapan semula tulang. Sebaliknya, jarak masa menopaus, pendidikan, bilangan penyakit, aktiviti fizikal, berat, BMI, ukur lilit pinggang, IGF1, DAL-SNPs dan kesan interaksi DAL-CMS tidak dikaitkan dengan penyerapan semula tulang.

Kesimpulannya, patofisiologi osteopororis dan kadar kehilangan tulang telah disedia maklum bahawa ia adalah satu proses yang rumit, dengan pelbagai faktor turut memainkan peranan. Dengan pertimbangan ini, kajian semasa memberi satu perspektif baru kepada kajian santifik ke atas faktor-faktor risiko untuk osteoporosis dalam kalangan wanita Cina yang sudah menopaus. Adalah sangat penting untuk penyelidik menggariskan korelasi-korelasi di antara beban asid dalam pemakanan, ciri-ciri CMS dan faktor-faktor genetik dengan penyerapan semula tulang dalam kajian-kajian lain. Kajian-kajian akan datang perlu meneroka bagaimana calon-calon SNP lain seperti reseptor vitamin D (VDR), beta reseptor estrogen (ERβ) dan pertukaran faktor tumbesaran-β1 (TGF-β1) mungkin berkorelasi dengan risiko penyerapan semula tulang dalam kalangan wanita pada peringkat pasca-menopaus.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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25(OH)D	25-dihydroxyvitamin D
AHA/NHLBIA	American Heart Association/National Heart, Lung, and Blood Institute
ALP	Alkaline phosphatase
BA	Bone area
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BSALP	Bone-specific alkaline phosphatase
BTM	Bone turnover markers
BUA	Broadband ultrasound attenuation
Ca^{2+}	Calcium ion
C/EBPα	CCAAT-enhancer-binding proteins
Cl^-	Chloride ion
CB-SEM	Covariance-based Structural Equation Modelling
CMS	Cardiometabolic syndrome
(COLIA1) Sp1	Alpha 1 chain of type 1 collagen
CRP	C-reactive protein
CTX1	C-terminal telopeptides of type I collagen
CVD	Cardiovascular disease
DAL	Dietary acid load
DBP	Diastolic blood pressure
DKK1	Dickkopf related protein 1
DPD	Deoxypyridinoline
DV	Dependent variable
DXA	Dual energy X-ray absorptiometry
f^2	Effect size

FBG	Fasting blood glucose
FPG	Fasting plasma glucose
FFQ	Food Frequency Questionnaire
FN	Femoral neck
FRAX	Fracture Risk Assessment Tool
GH	Growth Hormone
GPAQ	General Physical Activity Questionnaire
H+	Hydrogen ion
HbA1c	Hemoglobin A1c
HDL-C	High-density lipoproteins cholesterol
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HR-pQCT	High resolution peripheral quantitative computed tomography
HRT	Hormone replacement therapy
IDF	International Diabetes Federation
IQR	Interquartile range
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IGF-1	Insulin-like growth factor-1
IL-6	Interleukin-6
IL-1β	Interleukin-1β
IOF	International Osteoporosis Foundation
IPAQ	International Physical Activity Questionnaire
ISCD	International Society for Clinical Densitometry
IV	Independent variable
K^+	Potassium ion
LDL-C	Low-density lipoprotein cholesterol
LPL	Lower lipoprotein lipase
LS	Lumbar spine

MANS	Malaysian Adult Nutrition Survey
mEq	Milliequivalent
Mg^{2+}	Magnesium ion
MLR	Multiple linear regression
Na^{+}	Sodium ion
NaCI	Sodium chloride
NACSCOM	National Council of Senior Citizens Organisations Malaysia
NAFLD	Non-alcoholic fatty liver disease
NCEP	National Cholesterol Education Program
NEAP	Net endogenous acid production
NS	Not significant
NTX-1	N-terminal telopeptide of collagen type I
OC	Osteocalcin
OPG	osteoprotegerin
oz	Ounce
PA	Physical activity
PKU	Phenylketonuria
PLS-SEM	Partial least squares structural equation modeling
PO_4^{-}	Phosphate ion
pQCT	Peripheral quantitative computerized tomography
PRAL	Potential renal acid load
Pro:K	Potassium intake ratio
PSQI	Pittsburgh Sleep Quality Index
PTH	Parathyroid hormone
PYD	Pyridinoline
QCT	Quantitative computerized tomography
QUS	Quantitative ultrasound

R^2	Coefficient of determination
RANK	Receptor activator of NF-κB
RANKL	Receptor activator of nuclear factor kappa B ligand
RAPA	Rapid Assessment of Physical Activity
RCT	Randomized controlled trial
RNAE	Renal net acid excretion
PPAR-γ	Peroxisome proliferator-activated receptor-γ
SAMP6	Senescence-accelerated mouse P6
SBP	Systolic blood pressure
SEM	Structural Equation Modelling
sFRP-1	Secreted frizzled-related protein 1
SNP	Single nucleotide genetic polymorphism
SO_4^-	Sulfate ion
TC	Total cholesterol
TG	Triglyceride
TGF-β1	Transforming growth factor-β1
TNF-α	Tumor necrosis factor-α
WHO	World Health Organization
VDR	Vitamin D receptor
VIF	Variance inflation factor
WC	Waist circumference

CHAPTER 1

INTRODUCTION

1.1 Background of study

Osteoporosis is an age-related chronic disease characterized by low bone mass and deterioration of bone quality which affects more than 200 million people worldwide (Sugimoto et al., 2016). Compare to men, women are more susceptible to osteoporosis with 80% of osteoporosis patients being women, especially postmenopausal women (Al-Daghri et al., 2014; Al-Daghri et al., 2017). The sharp decline of estrogen during postmenopausal may reduce the rate of bone formation and increase bone turnover (Al-Daghri et al., 2014), leading to higher risk of hip fracture. In light of the demographic shift towards elderly population, 50% of all hip fractures are estimated to occur in Asia by 2050 (Lau et al., 2001), with a 2.28-fold increase of hip fracture incidence from 2018 to 2050 (Cheung et al., 2018). In a multi-ethnicity country like Malaysia, Chinese women were reported to have the highest prevalence of hip fracture of all ethnicities (Chan et al., 2020). Inadequate dietary calcium intake in the Chinese diet (Huang et al., 2018) and other unknown genetic variations (Chan et al., 2020) may have contributed to this.

Ample evidence show aging degenerative process is the major reason leading to cardiometabolic syndrome (CMS) (Bonomini, Rodella, & Rezzani, 2015; Chew, Ghazali, Ismail, Haniff, & Bujang, 2013; Dominguez & Barbagallo, 2016; Guarner-Lans, Rubio-Ruiz, Pérez-Torres, & de MacCarthy, 2011). Cardiometabolic syndrome is a combination of metabolic factors that increase the risk of cardiovascular disease and type 2 diabetes. Metabolic factors include hypertension, dyslipidemia, hypertension and abdominal obesity (Govindarajan, Whaley-Connell, Mugo, Stump, & Sowers, 2005). Cardiometabolic syndrome is prevalent among postmenopausal women, with an estimation 40% to 50% of postmenopausal women will have CMS (Lamb et al., 2011). Several researchers have reported that women, who have high risk of osteopenia and osteoporosis, have higher risk of CMS (Eckstein et al., 2016; Kim et al., 2013; Lamb et al., 2011).

Cardiometabolic syndrome and osteoporosis are multifactorial diseases that resulted from the interaction between environment and genetic factors. Environment factors such as low body mass index (BMI), smoking, sedentary life style, alcohol and caffeine intake, inadequate calcium and vitamin D intake, steroids, thyroid hormones and frusemides medicines for chronic diseases, serum ferritin level and endocrine diseases may associate with osteoporosis risk (Heidari et al., 2015; Loh & Shong, 2007). Current evidence shows that excessive dietary acid load (DAL) is a risk factor for osteoporosis and CMS through excess acid supply (Iwase et al., 2015; Jehle, Hulter, & Krapf, 2013; Mozaffari, Namazi, Larijani, Bellissimo, & Azadbakht, 2019).

In general, DAL can be categorized into acid load diet and base load diet. Acid load diet refers to diet high in animal sources but low in vegetable and fruit. It generates anions (acidic ions such as phosphate (PO_4^-), sulfate (SO_4^-), chloride (Cl^-) and organic acids) in the body, causing metabolic academia and increase urinary calcium excretion, which cause adverse effect on bone health (Fenton et al., 2009). On the other hand, base load diet is diet high in vegetables and fruits which may release cations (base ions such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+})) that compensate the excess acid ions and regulate the acid base homeostasis, decrease urinary calcium excretion and exert a protective effect on bone (Fenton et al., 2009). Cross-sectional studies and randomized controlled trial on DAL and low bone density (de Jonge et al., 2017; Jehle, Hulter & Krapf, 2013; Jia et al., 2015; McLean et al., 2011; Shi et al., 2012) had shown inconsistent findings. While there is inconclusive evidence on the relationship between DAL and bone health among Caucasian population, there is scarce of information on habitual intake of acidic or base diet among Malaysian Chinese. In spite of this, earlier local study showed a change of dietary pattern from traditional staple food such as rice, sweet potatoes, cassava, pulses and oilseeds to westernization of diet that high in meat, wheat-based products, processed foods, fats and oil, and sweeteners among Malaysian (Soon and Tee, 2014). Another local study demonstrated that Malaysian Chinese (159 g/day) has lower consumption of fruits and vegetables as compared to Malays (202 g/day) but slightly higher than Indians (126 g/day) (Izzah et al., 2012).

Genetic factors play an important role in the development of multifactorial diseases. A meta-analysis conducted by Yan et al. (2015) showed that interleukin-6 (IL-6) single nucleotide genetic polymorphism (SNP) was associated with reducing BMD and lead to osteoporosis. Lower insulin-like growth factor-1 (IGF-1) and elevated proinflammatory cytokine IL-6 may increase atherosclerosis progress (Sukhanov et al., 2007), osteoporosis (Yan et al., 2015) and CMS (Perez et al., 2014; Succurro et al., 2008). On the other hand, Schulze et al. (2005) identified a high intake of sweetened soft drinks, refined grains, red and processed meats as well as low consumption of wine, coffee, cruciferous vegetables and yellow-colored vegetables were positively correlated with inflammatory markers (included IL-6) and exert a modest effect on type 2 diabetes.

1.2 Problem statement

Despite acid base balance theory hypothesized habitual consumption of high acid load diet and inadequate consumption of potassium and bicarbonate-rich (base load diet) is associated with increase urinary calcium and magnesium loss and consequently a higher risk of osteoporosis, the evidence of the influence of DAL on bone health has been inconsistent. The contradictory findings may due to lacking of consideration between the links of DAL with diabetes (Fagherazzi et al., 2014), hypertension (Bahadoran, Mirmiran, Khosravi & Azizi, 2015), obesity (Bahadoran, Mirmiran, Khosravi & Azizi, 2015), cardiovascular disease (Akter et al., 2017), stress hormone cortisol (Krupp, Shi, Maser-Gluth, Pietzarka, & Remer, 2013) and overall well-being on bone health. The underlying mechanism explaining these associations have not yet been completely clarified.

Evidence is growing that nutrition transition leads to the deterioration of dietary quality. Changes in food production systems on processed meats, low nutrient dense foods, changes in food distribution and increased marketing by international food companies are among the reasons contributing to nutrition transition to poor dietary quality especially in low- and middle-income countries (Ronto, Wu, & Singh, 2018). As a result of easy accessible of poor dietary quality foods, low- and middle-income countries have adapted Western diets which are widely defined as acid load foods characterized as high in refined grains, added sugars and animal-source foods (Gaziano, Bitton, Anand, Abrahams-Gessel, & Murphy, 2010). Several studies regarding DAL estimation were available among Caucasian but such studies are scarce in Chinese population which has different culture and dietary habits than Caucasian. To the best of knowledge, there is no study on DAL among Malaysian, neither availability of study on the association between DAL and bone health.

In view of genetic perspective, recent evidence suggested that carries with IL-6 (-174G/C and -572G/C) CC genotype had higher risk of bone loss (Deveci, Ozkan, & Yuce, 2012; Ferrari, Ahn-Luong, Garnero, Humphries, & Greenspan, 2003; Ferrari et al., 2001; Garnero et al., 2002) while carriers with IGF-1 rs35767 CC genotype had higher BMDs at the total hip, trochanter, femoral neck and lumbar spine compared with TT and CT genotypes (Chen et al., 2017). It is however remains unknown whether the interaction between DAL and gene polymorphism could predict the risk of osteoporosis. Besides, gene association studies had identified IL-6 and IGF-1 polymorphism gene are responsible on metabolic traits (Boeta‐Lopez et al., 2018; Ma et al., 2016; Vaessen et al., 2001; Zhang, Chen, Zhang, & Peng, 2017). Along with the growing evidence on IL-6 and IGF-1 genetic polymorphisms and metabolic traits, there is increasing concern over the interaction effect of CMS and SNPs in predicting osteoporosis. Taken together, it is hope that this study is able to delineate the potential role of DAL, CMS and SNPs of IL-6 and IGF-1 on risk of osteoporosis and provide new insight for scientific benefits.

1.3 Research questions

The present study seeks to address the following questions:

1. What is the dietary acidity level, serum vitamin D status, CMS traits and bone resorption rate among postmenopausal women?
2. Are there significant relationships between sociodemographic background, lifestyle factors, anthropometry parameters and biochemical indices with risk of osteoporosis?
3. Is there any significant relationship between DAL and risk of osteoporosis?
4. Is there any interaction effect between DAL with IL-6 and IGF-1 genetic polymorphisms in predicting risk of osteoporosis?
5. Is there any significant interaction effect between DAL and CMS on risk of osteoporosis?
6. Is there any significant relationship between CMS and risk of osteoporosis?

7. Is there any interaction effect between CMS with IL-6 and IGF-1 genetic polymorphisms in predicting risk of osteoporosis?

1.4 Significance of study

This study develops the first DAL, CMS and genetic model of osteoporosis risk in postmenopausal Chinese women in Malaysia. Therefore, this study helps to extend the understanding of DAL, CMS and genetic factors by showing that the DAL and CMS model is able to explain the osteoporosis risk. The finding of this study would contribute to the literature by exploring another mechanism underlying the relationship between DAL and osteoporosis risk, aside from the known factors such as lower vitamin D, aging, being women, physical inactivity, and estrogen deficiency. The findings of this study contribute to the literature about whether there is moderating role of IL6 and IGF1 genetic polymorphisms in the relationship between DAL and osteoporosis, and also the relationship between CMS and osteoporosis risk. This study also provided an important opportunity to advance the understanding of interaction effect between CMS and genetic polymorphism on osteoporosis risk.

Over time, roles of high protein intakes on bone health is still inconclusive. While high protein diet may enhance calcium absorption, elevated circulating IGF1 and decreased serum PTH that may improve bone health (Cao & Nielson, 2010), increase of protein intake may create an acidic environment which may have revert effect on bone health. Thus, it is important to know the positive or negative pathway between DAL and bone health, or it is modest effect of high protein diet together with sufficient alkalinizing fruits and vegetables to enhance bone health. To date, there is limited DAL data among Chinese population and there is no DAL study among Malaysian Chinese. This study is significant to contribute the DAL data to Asian literature.

Evidence remain inconsistent on the potential association between DAL and bone health. One of the reasons may attributed to previous studies on DAL have not dealt with the interaction effects between DAL with genetic and environmental factors. This is the first study to take into account of interaction effects between DAL with genetic polymorphisms and CMS. The findings of whether high DAL with genetic polymorphisms and CMS risk may increase the risk of bone resorption, implied the dietary strategies for the bone health management.

Current body of literature mainly focus on DAL and BMD which was measured by DXA (Liu et al., 2015; Mangano et al., 2014; Pedone et al., 2010) but lacking of bone quality studies that was measured by bone turnover marker. As bone strength is determined by bone quantity and bone quality, BMD studies do not account for the bone mineral lost. Besides, there is different bone mass in different skeletal and therefore it creates low precision to diagnose osteoporosis. This dissertation served the limitation of BMD studies to bring clarity to an otherwise chaotic body of research.

Taken together, it is hope that this study is able to delineate the potential role of DAL, CMS and genetic polymorphism on risk of osteoporosis and provide new insight for scientific benefits. This study will provide critical information for the relevant authorities in planning, implementation, monitoring and evaluating of appropriate program to prevent or delay the onset of osteoporosis among the postmenopausal women.

1.5 Objectives of study

1.5.1 General objective

The study aimed to investigate the relationships between dietary acid load, genetic factors and cardiometabolic syndrome on risk of osteoporosis among postmenopausal Chinese women in Selangor and Kuala Lumpur, Malaysia.

1.5.2 Specific objectives

1. To estimate DAL, vitamin D status, CMS traits and bone resorption rate among postmenopausal women.
2. To examine the relationships between sociodemographic background, lifestyle factors, anthropometry parameters and biochemical indices with risk of osteoporosis.
3. To investigate the direct and interaction effects of DAL and SNPs (IL-6 and IGF-1) in predicting risk of osteoporosis.
4. To examine the direct and interaction effects of DAL and CMS in predicting risk of osteoporosis.
5. To determine the direct and interaction effects of CMS and SNPs (IL-6 and IGF-1) in predicting risk of osteoporosis.
6. To develop the diet, CMS and genetic model on risk of osteoporosis among postmenopausal women.

1.6 Alternative hypothesis

The alternative hypothesis of this study were:

H1: There are significant relationships between DAL, vitamin D status and CMS traits with bone resorption among postmenopausal women.

H2: There are significant relationships between sociodemographic background, lifestyle factors, anthropometry parameters and biochemical indices with bone resorption marker (serum collagen type 1 cross-linked C-telopeptide: CTX1).

H3: IL6 -572G/C, IGF1 rs35767 and IGF1 rs7136446 moderate the relationship between DAL and CTX1.

H3a: IL6 -572G/C moderates the relationship between DAL and CTX1.
H3b: IGF1 rs35767 moderates the relationship between DAL and CTX1.
H3c: IGF1 rs7136446 moderate the relationship between DAL and CTX1.

H4: There is significant relationship between DAL and CMS with CTX1.

H4a: There is significant relationship between DAL and CTX1.
H4b: There is significant relationship between CMS and CTX1.

H5: IL6 -572G/C, IGF1 rs35767 and IGF1 rs7136446 moderate the relationship between CMS and CTX1.

H5a: IL6 -572G/C moderates the relationship between CMS and CTX1.
H5b: IGF1 rs35767 moderates the relationship between CMS and CTX1.
H5c: IGF1 rs7136446 moderate the relationship between CMS and CTX1.

H6: There are significant relationships between DAL, CMS and genetic model on CTX1.

1.7 Operational definitions of study variables

1.7.1 Serum C-terminal telopeptides of type I collagen (CTX1) (Dependent variable)

Serum C-terminal telopeptides of type I collagen is the biomarker in serum to measure the rate of bone turnover. It has been recommended as bone resorption marker by International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (Vasikaran et al., 2011).

1.7.2 Dietary acid load (DAL) (Independent variable)

Diet acid load is defined as residual or excess hydrogen ion (H^+) production post food metabolism (Fenton et al., 2011). Generally, high dietary protein and grain food intakes may generate anions in the body and thus they are categorized as acidic diet (Fenton et al., 2009).

1.7.3 Cardiometabolic syndrome (CMS) (Independent variable)

Cardiometabolic syndrome is a cluster of metabolic dysfunction characterized by insulin resistance and impaired glucose tolerance, hypertension, dyslipidemia and abdominal adiposity (Mohamud et al., 2011). There are various diagnostic criteria

with variable cut-off values to define CMS. Continuous information of single indicators (blood pressure, waist circumference, triglyceride, HDL and fasting blood glucose) to define composites of CMS was used as independent variable.

1.7.4 Cardiometabolic syndrome (CMS) (Moderator)

Definition was discussed in section 1.7.3. In Malaysia, the International Diabetes Federation (IDF) (2005) and Harmonized definition (2009) are the most widely used diagnostic criteria for CMS. Individuals are to be diagnosed with CMS when they have any three or more out of five CMS criteria. Categorical cutoffs were used as moderator.

1.7.5 Interleukin 6 -572 G/C single nucleotide polymorphism (IL6 -572 G/C SNP) (Moderator)

Interleukin 6 is a pro-inflammatory cytokine that may play an important role in regulating bone resorption and increase estrogen deficiency-related bone loss in postmenopausal women. It may act both directly and indirectly to increase bone resorption and inhibit bone formation (Al-Daghri et al., 2014). IL6-572 G/C polymorphism is a candidate gene to reduce BMD and lead to osteoporosis (Yan et al., 2015).

1.7.6 Insulin-like growth factor-1 rs35767 single nucleotide polymorphism (IGF1 rs35767 SNP) (Moderator)

Insulin-like growth factor-1 (IGF1) is a single-chain polypeptide encoded by IGF1 gene. It is an important growth hormone and play a major role in regulating bone homeostasis and bone growth throughout adult life. IGF1 rs35767 polymorphism was reported to have significant association with risk of osteoporosis in Chinese postmenopausal women (Gao, Lv, Zhou, Mao & Sheng, 2018).

1.7.7 Insulin-like growth factor-1 rs7136446 single nucleotide polymorphism (IGF1 rs35767 SNP) (Moderator)

Definition was discussed in section 1.7.6. IGF1 rs7136446 is a potential predictor for fracture healing (Zeckey et al., 2011).

1.8 Conceptual framework

Figure 1.1 demonstrates the conceptual framework of the study. In this conceptual framework, the antecedent variables are age (Tian et al., 2017), years of education (Heidari et al., 2015), marital status (Pluskiewicz et al., 2014), monthly household income (Yoo & Park, 2018), number of diseases (Wong, Chin, Suhaimi, Ahmad, &

Ima-Nirwana, 2016), physical activity (Segev, Hellerstein, & Dunsky, 2018), weight (Wu & Du, 2016), height (Armstrong et al., 2016), serum 25(OH)D (Venugopal et al., 2017) and sleep quality (Ochs-Balcom et al., 2019) while bone resorption marker is the dependent variable of study.

DAL is intended to have a direct relationship with osteoporosis risk (Stroup et al., 2017). Bone is an organ that store most of the base ion in the form of alkaline salts of calcium (e.g., calcium hydroxyapatite, calcium carbonate). The acid-base balance theory hypothesized that bone may release base ion to neutralize part of the net dietary acid-ash in the body. Frassetto and colleagues (2018) suggested that older adults may have poorer renal function and decreased renal acid buffering ability, are benefits the most from base load diet (Frassetto, Banerjee, Powe, & Sebastian, 2018). If the acid-base balance theory is true, alkaline diet mainly from fruits and vegetable is important to prevent bone loss. However, evidence on acid-base balance theory is inconsistent.

IL6 (Fajar & Azharuddin, 2017), IGF1 (Gao et al., 2018) genetic polymorphisms and CMS (Zhou et al., 2013) that were purported to have association with osteoporosis risk were introduced into this relationship between DAL and CTX1. According to Hair et al. (2017), moderator variables are typically introduced when there is an unexpectedly weak or inconsistent relation between a predictor (DAL) and dependent variable (CTX1).

Literature on CMS components and bone health have produced inconsistent findings. For example, increased body weight has protective effect on bone health and decreased fracture risk (De Laet et al., 2005). However, increased abdominal adiposity positive correlates with osteoporosis and fracture risk (De Laet et al., 2005). Moreover, type 2 diabetic women showed higher risk of bone fracture; but when together with hyperinsulinemia, type 2 diabetic women showed higher BMD (Barrett-Connor & Kritz-Silverstein, 1996; Zhou et al., 2013). In addition, there are contradictory findings between dyslipidemia and osteoporosis (Adami et al., 2004; Yamaguchi et al., 2002). Similarly, there was inconsistent findings between hypertension and bone loss (Hanley et al., 2003). Therefore, CMS components were acted as independent variables and IL6 and IGF1 genetic polymorphisms were introduce into this relationship to examine whether they could strengthen or weaken the relationship between CMS components and CTX1. Lastly, an overall model between DAL, CMS and genetic polymorphisms on risk of osteoporosis are expected to be developed in the present study.

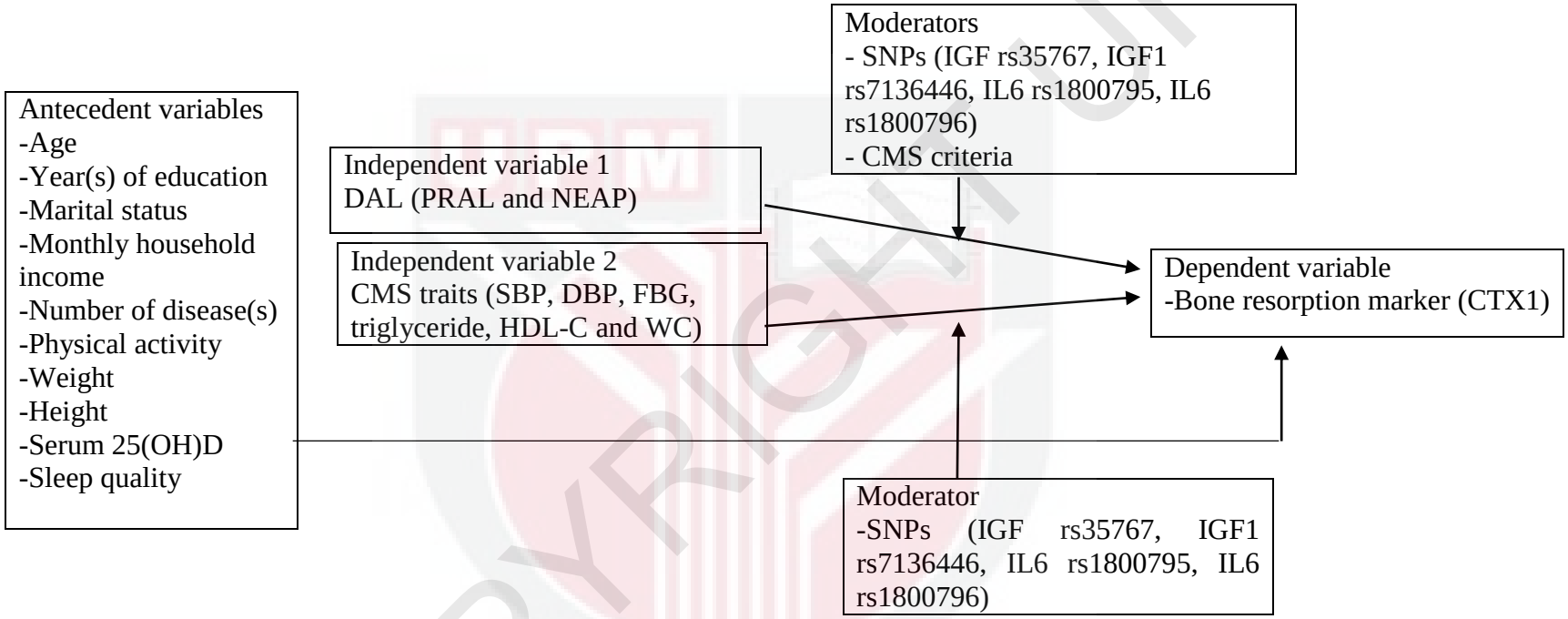

Figure 1.1 : Conceptual framework of the study

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