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Blood Inflammatory Markers, Anabolic Hormone, Vitamin D and L-Carnitine Status According to Frailty Status among Older Adults

(Penanda Inflamasi Darah, Hormon Anabolik, Vitamin D dan Status L-Karnitin menurut Status Keuzuran dalam Kalangan Warga Emas)

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ABSTRACT

Frailty is associated with functional decline and reduced ability to cope with physiological challenges. The pathophysiological changes in frailty include inflammation, endocrine disturbances, neuromuscular and immune dysfunction. However, comprehensive information on the relationship between biological markers and frailty is scarce. We aimed to determine the relationship between selected biomarkers and frailty among Malaysian older adults. In this cross sectional study, 600 community dwelling older adults were randomly selected from ten different areas in Klang Valley. A total of 382 participants met the inclusion criteria and consented to the study. Out of these, a subsamples of 91 participants were selected for biomarkers analysis. Frailty was defined using Fried's criteria. Fasting blood glucose, lipid profile, albumin, selected frailty biomarkers including IL-6, TNF- α and IGF-1 and vitamin D together with free L-carnitine and acetyl L-carnitine were analysed. The results showed a significantly higher level of serum IL-6 and TNF- α among participants classified as frail as compared to pre-frail and robust ($p < 0.05$). With respect to nutritional biomarkers, binary logistic regression showed that vitamin D deficiency is associated with three times higher risk of frailty ($p < 0.05$). There was no significant relationship between blood L-carnitines and frailty. Further research is required to explore the relationship between L-carnitine and frailty using different parameters involved in L-carnitine homeostasis such as CPTII enzyme and OCTN2 mutation. In conclusion, frailty was associated with increased level of inflammatory markers and vitamin D deficiency. It is empirical to promote healthy lifestyle or disease management to reduce inflammation and increase vitamin D status as one of the frailty prevention action plan among older adults.

Keywords: Anabolic hormone; frailty biomarkers; inflammatory mediators; L-carnitine; vitamin D

ABSTRAK

Keuzuran adalah berkaitan dengan kemerosotan status kefungsi dan keupayaan dalam menangani cabaran fisiologi. Perubahan patofisiologi keuzuran termasuklah ketakfungsi ototneuro, ketakupayaan fungsi imun, inflamasi dan gangguan endokrin. Namun begitu, tidak banyak penyelidikan yang mengkaji hubungan antara petunjuk biologi dan keuzuran secara menyeluruh. Oleh itu, kajian ini menentukan hubungan antara petunjuk biologi tertentu dan status keuzuran dalam kalangan warga emas. Seramai 600 orang warga emas daripada komuniti dipilih secara rawak dari 10 buah kawasan di Lembah Klang. Seramai 382 peserta memenuhi kriteria kajian dan bersetuju mengambil bahagian. Seramai 91 subjek dipilih daripada sampel keseluruhan untuk analisis petunjuk biologi. Keuzuran didefinisikan daripada kriteria Fried. Petunjuk biokimia keuzuran yang dikaji termasuk IL-6, TNF- α dan IGF-1 serta vitamin D, bersama-sama dengan L-karnitin bebas dan asetil L-karnitin dianalisis. Keputusan menunjukkan aras plasma darah IL-6 dan TNF- α adalah lebih tinggi secara signifikan dalam kumpulan uzur berbanding, prauzur dan robus ($p < 0.05$). Berhubung dengan petunjuk biologi pemakanan, analisis logistik binari menunjukkan kekurangan vitamin D berkaitan dengan risiko keuzuran sebanyak tiga kali ganda ($p < 0.05$). Tiada hubungan signifikan antara L-karnitin dalam plasma darah dengan keuzuran, oleh itu, penyelidikan lanjut diperlukan untuk mengkaji hubungan antara homeostasis L-karnitin seperti enzim CPTII dengan mutasi OCTN2. Kesimpulannya, keuzuran dikaitkan dengan peningkatan tahap penanda inflamasi dan kekurangan vitamin D. Terdapat keperluan untuk meningkatkan strategi promosi gaya hidup sihat atau pengurusan penyakit untuk menurunkan tahap keradangan dan meningkatkan aras vitamin D bagi mencegah keuzuran dalam kalangan warga emas.

Kata kunci: Keuzuran; L-karnitin; pengantara inflamasi; petunjuk biologi; vitamin D

INTRODUCTION

Frailty is a distinct clinical syndrome with a biological basis (Lang et al. 2010). It is defined as loss of ability to adapt to stress as a result of diminished functional reserves

(Weiss 2011) and declined ability to maintain homeostasis (Byard 2015). The pathogenesis of frailty involves numerous physiological adaptive response that include inflammation, neuromuscular dysfunction, endocrine

dysregulation, immune dysfunction, abnormalities in energy metabolism (Crensil 2010) and central nervous system failure (Mocchegiani et al. 2010).

Screening for frailty among older adults in clinical practice is limited probably due to the time consuming methods of doing so using standard validated tools (Annweiler et al. 2011). It has been suggested that the identification of blood markers that distinguish at-risk frail older adults would be useful for the purpose of both prevention and etiological studies (Collerton et al. 2012). In addition, evidence suggested that physiological changes are obvious in preclinical stage of frailty (prefrailty). It is hypothesized that these alterations are responsible for the characteristics that are used to classify the syndrome (Shamsi et al. 2012).

Understanding the mechanisms of aging and age-related disorders helps in comprehending frailty pathophysiology mechanism (Chung et al. 2009). The available evidence strongly suggests that the interactions between inflammatory pathway, endocrine regulation, mitochondria function, nutrition and genes are very important in molecular and physiological changes that contribute to the development of frailty in older adults (Mocchegiani et al. 2010). Based on the suggested mechanisms and etiology of frailty and related pathophysiological changes, several biological markers have been proposed as frailty biomarkers (Kumar et al. 2014).

The relationship between inflammatory mediators and oxidative stress with frailty, mainly Interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), C-reactive protein (CRP), homocysteine and oxidative stress has been examined (Lai et al. 2014). Significant relationships were reported with higher level of IL-6 (Almeida et al. 2012; Collerton et al. 2012; Lai et al. 2014), whilst the relationships with higher level of CRP and TNF- α were controversial. Other frailty biomarkers that have been studied are anabolic hormone IGF-1 (Leng et al. 2009); immune function (Collerton et al. 2012); micronutrients mainly vitamin D (Chang et al. 2010; Vogt et al. 2015); vitamin E (Ble et al. 2006); presence of heavy metal toxicity (García-Esquinas et al. 2015); visceral protein (Collerton et al. 2012; Hubbard & Woodhouse 2010); multisystem function kidney, liver function and lipid profile (Fernández-Garrido et al. 2014); and telomere length (Telomeres are repetitive DNA sequences at the ends of the chromosomes that protect the DNA against degradation and maintain chromosomal stability) (Saum et al. 2014). Yet, there is no gold standard biomarker for frailty that can be used to predict and prevent frailty, or used in etiological studies (Annweiler et al. 2011).

L-carnitine is a non-essential nutrient, fundamental to cellular energy metabolism, synthesized in the liver, kidney and brain through the conversion of two essential amino acids, lysine and methionine (Crensil 2010). L-carnitine deficiency is associated with persistent inflammatory state and immune dysregulation in or for some diseases (Campos et al. 1995). Crensil (2010) has proposed a

relationship between L-carnitine deficiency and frailty through several mechanisms including its effect on skeletal muscle metabolism, hyperinflammation, energy production and immune cells function. L-carnitine plays an obligatory role in mitochondrial function in oxidation of long-chain fatty acids. Serum fatty acid was found to be higher with increasing age, and the ratio of omega 3 to omega 6 fatty acid was significantly lower in older adults (Hall & Jewell 2012). This might explain the mild proinflammatory state often found in older adults (Hall & Jewell 2012).

In addition, L-carnitine deficiency may lead to frailty through mitochondria dysfunction manifested by weakness, sarcopenia and fatigue which are components of frailty. Another important point is that muscle weakness is a major characteristic of frailty. L-carnitine deficiency can affect skeletal muscle function by its role in beta oxidation, the main energy source for skeletal muscle (Crensil 2010). High serum L-carnitine levels were also found to be correlated with better functional abilities (Brass 2001). However, L-carnitine serum level has not been extensively studied among frail or prefrail compared to robust older adults. We aimed to determine the status of suggested frailty biomarkers such as IL-6, TNF- α , IGF-1 and vitamin D among the three groups of older adults (frail, prefrail and robust), and to examine the plasma level of L-carnitine as a potential biomarker for frailty.

MATERIALS AND METHODS

This study is a part of larger cross sectional study conducted in Klang Valley, Malaysia as reported earlier in Shahar et al. (2015). Ethical approval was obtained from Universiti Kebangsaan Malaysia Medical Research and Ethics Committee (UKM 1.5.5.5/244/NN-149-2013). Participants were selected by multistage random sampling from nine rural and urban districts, of which the percentage of older adults was 10% and above. More than 600 Malaysian older adults aged 60 years and above, were personally invited to participate in this study. A total of 574 respondents agreed to join the study and signed the consent form. The response rate was 92.5%. A total of 382 participants met the inclusion criteria and consented to provide their blood for biochemical analysis. Out of this figure, a sub samples of 91 participants were selected from the; frail, prefrail and robust groups. All of the respondents from the frail group were included, and the selected prefrail and robust groups were then matched for age, gender, educational level, economic status and ethnicity.

Frailty was defined using Fried's criteria which is widely used in clinical research and for frailty assessment (Rockwood et al. 2007). It was developed in a very large prospective study (Fried et al. 2001) involving 5000 elderly individuals, aiming to develop and validate a phenotype of frailty in older adults. Shrinking, exhaustion, weakness, slowness and low physical activity were concluded as the assessment criteria. Participants with three or more (≥ 3) criteria were defined as frail; with one or two (1 or 2) as prefrail; and with zero (0) as robust (Fried et al. 2001).

In order to obtain accurate and standard biochemical tests, the respondents were asked to fast overnight. Peripheral venous blood samples from the respondents were collected by a trained phlebotomist. A total of 20 mL blood was collected in tubes and kept in an ice box until it was transferred to the lab. Albumin, fasting blood sugars (FBS), glycosylated hemoglobin (HBA1c) and lipid profile were conducted at the Path Lab-Pathology and Clinical Laboratory, Sdn Bhd - Klang Valley branches - Malaysia, for quick analysis. The remaining blood, serum and plasma were aliquoted at the Path Lab, then transferred and stored in freezer at (-80°C) located at the food analysis Lab- Faculty of Health Sciences-UKM, for further analysis. Selected frailty biomarkers including IL-6, TNF- α and IGF-1 in addition to vitamin D were analyzed to determine their relationship with frailty. IL-6, TNF- α , IGF-1 and vitamin D were detected using the blood serum, while the free, total and the acetyl L-carnitine were analyzed using blood plasma.

The plasma level of L-carnitine and total L-carnitine were done using a validated method (Prieto et al. 2006). It is an easy and accurate method to measure the L-carnitine status in human plasma using spectrophotometric analysis. The chemicals were purchased from two companies: MERCK SDN BHD and Sigma SDN BHD. The chemicals from MERCK were L-carnitine for synthesis, Acetylcoenzyme A, Trillium salt, (70-72%) Perchloric acid, Potassium Hydroxide pellets for analysis, Hydrochloric acid, and Sodium Carbonate anhydrous for analysis. Disodium EDTA, 5,5'- dithio-bis (2-Nitrobenzoic acid) DTNB and 4-(2-hydroxyethyl) -1-piperazineethanesulfonic acid HEPES; while Carnitine acetyl transferase chemical was bought from Sigma. The steps of the analysis were done following Prieto et al. (2006) protocol. The L-carnitine insufficiency was defined by the value of free L-carnitine: acetyl L-carnitine ratio less than 0.4 as suggested by Calabrese et al. (2006). The detection of IL-6, TNF- α , IGF-1 and vitamin D in the serum was done using Enzyme-linked Immunosorbent Assays kit (R&D systems, USA). The samples and reagents preparation and assay procedures were done following the kits manual provided by the

company. Vitamin D deficiency was defined by value of 25(OH)D serum level (<25 nmol/l) (Vogt et al. 2015).

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 21.0. An alpha level of (0.05) was considered for all the statistical tests in the study. Two sided p values of (0.05) and (80%) power were considered to be statistically significant. Normality test showed that the data was not normally distributed. The mean differences between the three groups (frail, prefrail and robust) were tested using Kruskal Wallis test as the data was not normally distributed. For categorical variables, Chi square test was employed to determine the association between frailty status and the categorical variables (Vitamin D and L-carnitine deficiency). Binary logistic regression was done to determine frailty predictors. The multicollinearity, outliers and goodness of fit tests was employed to assess how well the model fit the data (Field 2013).

RESULTS AND DISCUSSION

The results showed that participants mean age was 68 \pm 5.9 years and there were no significant differences between the three groups in term of gender, age, race and economic status as shown in Table 1.

The mean of albumin, fasting blood sugars and lipid profiles were not statistically different between the three groups (frail, prefrail and robust), as shown in Table 2.

Regarding frailty biomarkers, some of the selected frailty biomarkers differed statistically between the three groups. The mean inflammatory mediators (IL-6 and TNF- α) were higher in frail as compared to prefrail and robust group ($p < 0.05$ for all parameters). Similar trend was found in IGF-1 but the differences did not reach a significant level. Vitamin D deficiency was higher in frail (85%), followed by prefrail (76.6%) and robust (54.5%) group ($p < 0.05$). L-carnitines plasma level showed no significant differences among the three groups and L- Carnitine insufficiency was detected in 21.1% frail, 19.8% prefrail and 20.1% robust group, with no significant difference between the three groups ($p > 0.05$) as shown in Table 3.

TABLE 1. Sociodemographic characteristics (Present as n (%))

	Factors	N	Robust (n=30)	Prefrail (n=32)	Frail (n=29)	P value
Gender	Men	43	14(33)	16 (37)	13 (30)	0.678
	women	48	16(33.3)	16(33.3)	16(33.3)	
Age	60-70	49	17 (35)	19(39)	13(26)	0.245
	>70	42	13 (31)	13(31)	16 (38)	
Ethnicity	Malay and Indian	40	12 (30)	16(40)	12(30)	0.517
	Chinese	51	18 (35)	16 (31)	17 (34)	
Marital status	Married	53	18 (34)	21(40)	14 (26)	0.18
	Other	38	12 (32)	11(29)	15(39)	
Monthly income	Low <1500 rm	35	11(31)	13 (38)	11(31)	0.079
	Moderate- high >1500 rm	56	19 (34)	19 (34)	18(32)	

TABLE 2. Biochemical profile according to frailty status and gender (presented as Mean±SD)

Tests	Unit	Total (91)		
		Robust (n=30)	Prefrail (n=32)	Frail (n=29)
FBG	mmol/L	6.00±1.6	6.4±2.5	5.6±0.71
Total cholesterol	mmol/L	5.2±1.1	5.2±1.1	5.1±0.9
HDL	mmol/L	1.5±0.3	1.4±0.4	1.4±0.6
LDL	mmol/L	3.2±0.9	3.1±0.9	2.9±0.8
Triglyceride	mmol/L	1.3±0.7	1.5±0.8	1.3±0.6
TC: HDL	---	3.8±1.0	3.9±1.2	3.5±0.9
Albumin	g/L	42.7±3.0	42.8±3.1	42.7±2.8

NS: no significant differences using Kruskal Wallis test.

Abbreviation: FBG: fasting blood glucose, HDL=high density lipoprotein, LDL=low density lipoprotein

TABLE 3. Frailty biomarkers and L-carnitine blood level, according to frailty status and gender (presented in Mean±SD)

Tests	Unit	Normal range	Robust (n=30)	Prefrail (n=32)	Frail (n=29)	P value
Interleukin-6 (IL-6)	pg/mL		5.4±2.2	6.2±2.8	10.6±5.4	0.000**
Tumor necrosis factor – alpha TNF-α	pg/mL		4.6±1.4	6.2±1.8	7.3±6.0	0.017*
Insuline like growth factor (IGF-1)	ng/mL	(47-190)	62.1±16.5	51.3±13.1	50.7±19.7	0.212
Vitamin D	ng/mL	>25	23.4±7.7	17.3±4.6	16.2±7.5	0.035*
Free Carnitine	µmol/L		33.3±2.1	32.1±9.2	33.6±2.9	0.197
Acetyl Carnitine	µmol/L		7.4±4.2	6.2±5.4	7.1±5.3	0.383
Total Carnitine	µmol/L		40.6±4.0	39.2±4.6	40.7±3.9	0.651
Free carnitine: Acetyl carnitine			0.226±0.13	0.281±0.36	0.22±0.18	0.585

*significant at p<0.05 using Kruskal Wallis test.

Abbreviations: IL-6: interleukin 6, TNF-α: tumor necrosis factor – alpha, IGF-1: Insuline like growth factor

Results from binary logistic regression indicated that vitamin D deficiency increased risk of frailty by three times, after adjustment for age and gender (OR 3.69, 95% CI, p<0.01), and higher level of Interleukin -6 increase the risk of frailty (OR 1.2, 95%, p<0.05), as shown in Table 4.

Blood indicators, such as albumin, lipid profile, fasting blood glucose and vitamin D, were assessed in the light of their relationship with frailty. Albumin was used in clinical setting to predict mortality in elderly and better health and nutritional status (Schalk et al. 2004) and associated with better functional performance in older adults (Villareal et al. 2004). However, in the current study, albumin blood level did not show significant relationship with frailty. The non-significant results might be due to normal albumin blood level in all of study subjects.

Similar to albumin, there were no significant differences in the means of the lipid profile and fasting blood glucose between the frail group and the others. However, there was a significant association between the lower level of high density lipoproteins (HDL) with being frail/prefrail; low blood level of HDL associated with double risk to develop frailty. Villareal et al. (2004) did not find any significant difference in the means of any of the lipid profile tests between frail and non-frail elderly. Fried

et al (2001) reported an association between frailty and cardiovascular diseases. This highlights the importance for conducting more research to identify the relationship between frailty and other chronic diseases and their impact on elderly health.

Vitamin D, as a frailty biomarker and nutritional status parameter, was found to be lower in frail and prefrail subjects as compared to robust. This is also reported by other researchers (Chang et al 2010; Puts et al. 2005; Vogt et al. 2015). Vitamin D has a primary physiological role in human body. It is involved in many metabolic processes and pathways (Mithal et al. 2009). In elderly, it was found that vitamin D status could predict the decline in the physical performance (Wicherts et al. 2007); and it was related to falls risk, fracture rate and rate of bone loss among the elderly (Dawson-Hughes et al. 2005). Frailty definition includes physical function impairment and metabolic changes. Similarly, vitamin D deficiency also leads to physical function impairment and metabolic changes (Vogt et al. 2015).

Frailty biomarkers reported in the literature including the inflammatory mediators (IL-6 and TNF-α) and the anabolic hormone (IGF-1) were determined and investigated in association with frailty. These biomarkers

TABLE 4. Frailty predictors from selected biomarkers

Factors	B	P value	Exp (B)	Exp (B)	P
Age	0.13	0.012*	1.12	2.63	0.000**
Gender	0.167	0.764	1.12		
Vitamin D <25 (ng/mL)	1.25	0.034*	3.69		
TNF α (pg/mL)	0.13	0.058	1.1		
IGF-1 (ng/mL)	-0.09	0.07	0.9		
IL-6 (pg/mL)	0.127	0.02	1.2		

Cox & Snell R square = 0.157 and Nagelkerke R square = 0.291

Abbreviations: IL-6: interleukin 6, TNF- α : tumor necrosis factor – alpha, IGF-1: Insulin like growth factor.

** Significant at $p < 0.01$ using Binary logistic regression test

were analyzed in a sub-sample from the total participants. The results showed a significantly higher serum level of IL-6 and TNF- α in frail subjects as compared to prefrail and robust. The level of the anabolic hormone IGF-1 was lower in frail compared to prefrail and robust, but it did not reach the significant level. Serum level of the inflammatory cytokines IL-6 was correlated with changes in body composition, disability and morbidity related to aging (Walston et al. 2005). In many other studies higher levels of IL-6 and other proinflammatory cytokines were reported in frail compared to robust and prefrail (Collerton et al. 2012; Hubbard & Woodhouse 2010; Walston et al. 2005) community dwelling older adults. Collerton et al. (2012) reported that IL-6 was a significant predictor of frailty status among the very old older adults aged 85 years and above. Similar findings were reported among institutionalized older adults in long term care facilities (Lai et al. 2014). All of these findings support the hyper inflammation theory of pathophysiological changes in frailty. Tumor necrosis factor – alpha (TNF- α), is another pro inflammatory cytokines, that usually increases with normal aging (de Gonzalo-Calvo et al. 2010), and in age related diseases like Alzheimer (Cheng et al. 2014). TNF- α has been studied in the light of its relationship with frailty, but the results were not consistent as in IL-6. Our study results also showed a significant higher serum level of the TNF- α in frail compared to prefrail and robust. This was contradictory to the reports in other studies that did not find a significant association between frailty and TNF- α (Collerton et al. 2012; Hubbard & Woodhouse 2010; Lai et al. 2014).

Changes in the hormones and hormone response in aging are documented and correlated with age related diseases (Ferrari & Magri 2008; Marzetti et al. 2013). Decline in anabolic hormones such as growth hormone and insulin like growth factor (IGF-1) is also documented. This decline was associated with sarcopenia, cognitive decline and other disorders (Jones & Boelaert 2015). Even though this study did not find a significant difference in the level

of IGF-1 among frail, prefrail and robust groups, there was a trend for its decline with frailty. Leng et al. (2009) found a significant association between frailty and low level of IGF-1 in older women with moderate to severe disability. Moreover, in a recent study conducted among outpatients' older men, a significant relationship with frailty was found (Mohammed & Khater 2015). The differences between the results of our study and previous studies is probably due to the difference in participants' selection, analysis methods and sample size.

The relationship between L-carnitine and frailty was proposed from the similarities between L-carnitine deficiency and pathophysiological changes that occurred in frailty, namely abnormalities in energy metabolism, inflammation and impaired in immune function (Crentsil 2010). L-carnitine supplementation improved muscle function (Rivero et al. 2002), decreased fatigue (Cavallini et al. 2004; Malaguarnera et al. 2007), improved body composition and regulated inflammation process (Pertosa et al. 2005). To the best of our knowledge, there have been no studies published in the searched databases reporting the relationship between L-carnitine and frailty. Even our study failed to provide evidence for the relationship between the L-carnitine and acyle carnitine with frailty. The non-significant results in our study may be due to the small sample size, majority of the participants been well nourished as micronutrients deficiency affects the ability of the body to synthesize L-carnitine. There is a need for further research to examine this relationship by including all the susceptible mechanisms that may involve in frailty such as CPTII enzyme and OCTN2 mutation or functional decline.

CONCLUSION

Frailty was associated with hyper inflammatory status as indicated by the significant higher level of serum IL-6 and TNF- α . Vitamin D deficiency increased the risk of frailty by three times. Chronic disease management, healthy lifestyle

behavior and diet are important to improve vitamin D status and inflammatory markers in order to prevent frailty among older adults.

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