Original Article

The Association between Urinary Malondialdehyde and Obesity

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Background: Malondialdehyde (MDA) is end-product of polyunsaturated fatty acid. Studies have highlighted MDA as a biomarker of whole-body oxidative stress.

Objective: To explore the association between urinary MDA and obesity status.

Materials and Methods: The present research was performed as a cross-sectional study at the Primary Care Unit of a tertiary care university hospital in southern Thailand. Data were collected via questionnaire, medical records, and urinary MDA sample measured with high-performance liquid chromatography. Data were analyzed by R program version 4.0.0.

Results: One hundred fifteen participants comprised of 57 obese and 58 non-obese patients were included in the present study. The means of body mass indexes (BMIs) were 27.5±2.3 versus 21.9±1.7 kg/m^2 in obese and non-obese group, respectively. The median of urinary MDA among obese was significantly higher than in non-obese participants at 5.7 (6.0) versus 3.6 (3.9) µg/mL, (p=0.016). Additionally, the urinary MDA in central obesity at 5.8 (5.9) µg/mL was higher than normal waist circumference at 3.5 (3.8) µg/mL, (p=0.009).

Conclusion: Urinary MDA was significantly higher in the obesity and central obesity group than the lower or normal BMI and waist circumference group. Obesity could be an essential factor promoting the body's oxidative stress. Since oxidative stress is well known to be involved in the causes of lifestyle-related or chronic diseases, this specific biomarker can be used to detect and monitor the development of these diseases.

Keywords: Biomarker; Malondialdehyde; Obesity; Oxidative stress; Urinary MDA

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Obesity is defined as excessive fat accumulation that proposes a metabolic disease[1]. The World Health Organization (WHO) has announced obesity as the most significant global chronic health problem in adults. Obesity is also associated with health problems that increase death from stroke, heart disease, and cancers[2]. It is a leading cause of death and disabilities worldwide, affecting adults, children, and adolescents. In clinical practice, clinicians use body mass index (BMI) and intra-abdominal fat accumulation assessed by waist circumference (WC) to assess body fatness[2,3]. According to WHO's data in 2016, more than 1.9 billion adults worldwide were overweight and over 650 million adults were obese[4].

Thai people health survey in 2014 found that more than 32.9% of males and 41.8% in females above 15 years old were diagnosed with obesity[5].

Oxidative stress implies a disproportion between oxidants and antioxidants, which can cause tissue injury[6]. Gabriele et al in 2017 showed that oxidative stress is a destructive process that can negatively affect tissue and cellular structures such as deoxyribonucleic acid (DNA), protein, lipids, and lipoproteins and can cause lipid peroxidation, thus, injuring cell membranes[7]. Oxidative stress can lead to causes of various diseases such as primary or secondary cardiovascular diseases (CVDs) or neurological diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, or mental illness such as depression[7-9]. Studies have found a higher level of oxidative stress levels in obese people, explained by mechanisms such as low-grade chronic systemic inflammation, hyperglycemia, or impairment of antioxidant defense systems[6,10].

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Malondialdehyde (MDA) is an end-product of polyunsaturated fatty acid peroxidation. It is comprehensively used as a biomarker or a reliable tool for assessing oxidative stress in the whole body[6,11,12]. It has been realized that urinary MDA level is associated with morbidities such as obesity, diabetes mellitus, and epilepsy[6,13-15].

The authors intended to determine the association between urinary MDA, the biomarker of oxidative stress, and obesity status, a common disease caused by physical inactivity and unhealthy diet. The urinary MDA is beneficial as a non-invasive test monitoring oxidative stress in the whole-body. Few studies have focused on this affiliation, especially in primary care in Thailand.

Material and Methods

Study setting and design

A cross-sectional descriptive study was conducted in two groups at the primary care unit (PCU) of Songkhlanagarind Hospital, a tertiary care university hospital in southern Thailand, between May and August 2020.

Study population

Participants were aged 35 years or older visiting the PCU of Songklanagarind Hospital. The subjects were divided into two groups. The obese group was formed of patients with a BMI of 25.0 kg/m² or more, using the Western Pacific Reginal Office (WPRO) criteria[16] or a WC of 90 cm or more in male or 80 cm or more in female. The non-obese group was formed from patients with a BMI of 18.5 to 24.9 kg/m² and a WC of less than 90 cm in male and less than 80 cm in female. Patients who could not collect the urine specimen, had incomplete laboratory data, had a history of taking antioxidants or any supplements within the last one month, and had lower urinary tract symptoms, were excluded.

The calculated sample size was 122 with 61 for the obese group and 61 for the non-obese group, calculated by following two independents mean formula, which is referenced from a previous study[6]

$$n_1 = \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 \left[\sigma_1^2 + \frac{\sigma_2^2}{r}\right]}{\Delta^2}$$

$$r = \frac{n_2}{n_1},\ \Delta = \mu_1 - \mu_2$$

μ_1=1.56, μ_2=2.08, σ_1=0.85, σ_2=1.16, r=1, α=0.05, β=0.2

Measurements

After informed consents were approved and WC and BMI were performed. The researchers recorded the patient's laboratory data by using the latest visit from the Hospital Information System (HIS) of Songkhlanagarind Hospitals database. The laboratory results, including fasting blood sugar (FBS), serum blood urea nitrogen (BUN), serum creatinine (Cr), plasma total cholesterol (TC), Low-density lipoprotein-cholesterol (LDL-C), High-density lipoprotein cholesterol (HDL-C), and triglycerides were collected. Finally, the researchers explained to the participants how to collect urine samples and gave urine collection equipment.

Urinary MDA

Urine samples of 40 mL were collected from all participants. To reduce confounder factors such as food intake, all urine sample was collected before noon on the day of the hospital visit. An aliquot of 10 mL was separated into another tube to determine the urinary creatinine by CREP2 (creatinine plus version 2). The remaining spot urine sample, 30 mL, was stored in a polypropylene tube and frozen at –80°C before preparation and analysis. Urinary MDA was measured with HPLC (1100 Series; Agilent, Foster City, CA, USA) with diode array detector (DAD), UV detector (310 nm for Excitation wavelength and 510 nm for Emission wavelength), and Agilent ZORBAX columns (4.6×250 mm ID, 5 μm particle size). The limit of detection of the method was 0.15 nmol/L. The recovery of MDA was 85% to 115% obtained by adding eight concentrations of standard solutions, from 0.1 to 50 μg/mL, to the urine samples. The reproducibility was 90% to 110%. The urinary MDA was expressed as μg/mL.

The variables

Variables were both independent and dependent variables. Independent variables included age, gender, smoking status, alcohol drinking status, exercise status, blood pressure, BMI and WC, blood lipid, FBS, BUN, Cr, and underlying disease. Dependent variables included oxidative stress parameter and urinary MDA.

Statistical analysis and ethical issue

Data analysis was performed by using R program 4.0.0. Continuous data were expressed as the mean ± standard deviation or median (interquartile range) depending on the distribution of data. Categorical data were expressed as frequencies or percentages. Chi-square test, Fisher's exact test, t-test, and Wilcoxon rank-sum test were used to test the difference in

Table 1. Characteristics of participants divided into the two study groups (n=115)

Variables	Non-obese: BMI <25.0 kg/m^2 (n=58)	Obese: BMI ≥25.0 kg/m^2) (n=57)	p-value
Sociodemographic parameters			
Age (years); mean±SD	64.2±9.2	60.0±9.1	0.017*
Sex; n (%)			0.025
• Female	30 (41.7)	42 (58.3)	
• Male	28 (65.1)	15 (34.9)	
Smoking status; n (%)			0.236
• Current	8 (72.7)	3 (27.3)	
• Never	38 (46.3)	44 (53.7)	
• Ex-smoker	12 (54.5)	20 (45.5)	
Alcohol status; n (%)			0.062
• Current	17 (70.8)	7 (29.2)	
• Never	32 (43.2)	42 (56.8)	
• Ex drinker	9 (52.9)	8 (47.1)	
Regular exercise#; n (%)			0.009
• Regular	30 (66.7)	15 (33.3)	
• Non-regular	28 (40.0)	42 (60.0)	
Anthropometric parameters; mean±SD			
BMI (kg/m^2)	21.9±1.7	27.5±2.3	<0.001**
Waist circumference (cm)	79.3±6.5	91.6± 6.9	<0.001*
Metabolic parameters (mg%); mean±SD			
LDL-cholesterol	117.6±27.7	121.9±37.1	0.962*
HDL-cholesterol	62.3±16.7	55.1±12.4	0.016*
Triglyceride	107.9±45.3	135.4±62.1	0.010*
Cholesterol	183.9±31.8	188.8±36.5	0.955*
FBS	102.1± 14.7	112.9 ± 44.8	0.231*
Underlying disease; n (%)			
HT+DLP	18 (37.5)	30 (62.5)	0.044
HT+DM	5 (71.4)	2 (28.6)	0.264***
HT+DM+DLP	6 (46.2)	7 (53.8)	1***
DLP+DM	20 (62.5)	12 (37.5)	0.101

SD=standard deviation; BMI=body mass index; FBS=fasting blood sugar; HDL=high density lipoprotein, LDL=low density lipoprotein; HT=hypertension; DLP=dyslipidemia; DM=diabetes mellitus

Aerobic exercise 150 minutes per week

p-value by Pearson chi-squared, * Wilcoxon rank-sum test, ** t-test, *** Fisher's exact test

independence variables between the obese and the non-obese group. Bivariate and multiple regression analysis were performed to investigate the association between factor and urinary MDA. For the relationship between independent variables and urinary MDA, a p-value of less than 0.05 was considered as the statistically significant.

The present study was approved by the Institutional Review Board of Prince of Songkla University (REC 63-141-9-1).

Results

One hundred fifteen participants who met inclusion criteria were divided into two groups, with 57 in the obese group and 58 in the non-obese group. Seventy-two females (62.6%) participated in the present study. The mean age of all the participants was 62.1±9.3 years, the mean age of non-obese group at 64.2±9.2 years-old was higher than the obese group at 60.0±9.1 years-old. Furthermore, BMI and WC of the obese group were higher than the non-obese at 27.5±2.3 versus 21.9±1.7 kg/m² and 91.6±6.9 versus 79.3±6.5 cm, respectively. HDL-cholesterol level in non-obese participant was higher than the obese (p=0.016), and triglyceride level in obese group was statistically higher than non-obese (p=0.010). There were no significant differences in smoking and alcohol consumption, LDL-cholesterol, cholesterol,

Table 2. Difference in urinary MDA among obesity group (n=115)

Variables	Urinary MDA (µg/mL); median (IQR)	p-value
Obesity by BMI group		0.016
Non-obesity group (BMI <25 kg/m^2)	3.6 (3.9)	
Obesity group (BMI ≥25 kg/m^2)	5.7 (6.0)	
Obesity grade		0.064
Normal (BMI 18.5 to 22.9 kg/m^2)	3.9 (4.1)	
Overweight (BMI 23.0 to 24.9 kg/m^2)	3.4 (2.5)	
Obesity grade 1 (BMI 25.0 to 29.9 kg/m^2)	6 (6.1)	
Obesity grade 2 (BMI ≥30.0 kg/m^2)	4.8 (3.4)	
Waist circumference		0.009
Normal (WC <90 cm in male, <80 cm in female)	3.5 (3.8)	
Central obesity group (WC ≥90 cm in male, ≥80 cm in female	5.8 (5.9)	

BMI=body mass index; IQR=interquartile range; MDA=malondialdehyde; WC=waist circumference

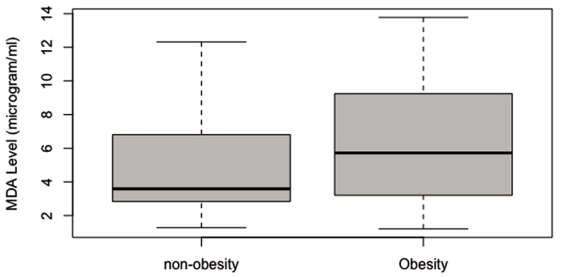

Figure 1. Boxplots between BMI and urinary MDA (n=115).

FBS, and underlying diseases between the non-obese and the obese groups (Table 1).

Urinary MDA level was categorized by BMI group for non-obese and obese group. The BMI grading is known as four groups by WPRO(16) criteria as normal, overweight, obesity grade 1, obesity grade 2, and WC as normal and central obesity. The results demonstrated that the median of urinary MDA level in the obese group at 5.7 (6.0) µg/mL was significantly higher than the non-obese group at 3.6 (3.9) µg/mL. Categorized by WCs, the result showed that urinary MDA level in central obesity at 5.8 (5.9) µg/mL was significantly higher than normal WC at 3.5 (3.8) µg/mL. However, urinary MDA level categorized by obese grading showed no statistically significance difference (p=0.064) (Table 2).

The boxplots illustrated the relationship between urinary MDA and BMI group. The results showed that the median MDA in obese group at 5.7 (6.0) µg/mL is higher than the median MDA in non-obese group at 3.6 (3.9) µg/mL, (p=0.016) (Figure 1).

Bivariate and multiple regression analysis were performed describing the relationship between sociodemographic parameters, anthropometric parameters, metabolic parameters, and biomarker of oxidative stress, using urinary MDA. Multiple regression analysis showed that BMI, cholesterol, and LDL variables related with urinary MDA. It was demonstrated that obese patients have a positive correlation with urinary MDA (β 1.66, 95% CI 0.49 to 2.82). Elevation in cholesterol level showed a positive correlation with MDA (β 1.44, 95% CI 0.03 to 2.84). In cases where there was elevation in the level of LDL-cholesterol (β –2.00, 95% CI –3.31 to –0.68), oxidative stress was significantly diminished on negative factors. There was no association between triglyceride and urinary MDA (Table 3).

Discussion

The authors investigated associations between obesity and urinary MDA using a cross-sectional descriptive design. The study gathered data were from participants in the primary care setting. MDA, the end-product of polyunsaturated fatty acid peroxidation, is comprehensively utilized as a biomarker or a reliable tool for assessing oxidative stress in the whole body[6,11,12]. Oxidative stress can lead to causes in various diseases such as primary or secondary CVDs or neurological diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, or mental illness such as depression[7-9]. From the present study, most baseline characteristics of both obese and non-obese groups were not different. However, the anthropometric parameters and the metabolic parameters, including BMI, WC, lipid profiles and FBS, in the obese group tended to be higher than in the non-obese group. These results were consistent with a previous study that found the lipid profiles positively correlated with BMI[17].

The urinary MDA levels were significantly

Table 3. Bivariate and multiple regression analysis between urinary MDA and factors

Variables	Bivariate		Multivariate		
	Coefficient	95% CI	Coefficient	95% CI	p-value
Constant			6.94	5.33 to 8.55	<0.010
Triglyceride (mg%)	0.00	−0.02 to 0.01	−0.01	−0.02 to 0.00	0.072
BMI group (ref.: non-obese <25 kg/m^2)					
Obese (≥25 kg/m^2)	1.60	0.44 to 2.77	1.66	0.49 to 2.82	0.006*
Cholesterol (ref.: <200 mg%)					
≥200 mg%	0.57	−0.80 to 1.93	1.44	0.03 to 2.84	0.046*
LDL-cholesterol (ref.: <100 mg%)					
≥100 mg%	−1.56	−2.80 to −0.31	−2.00	−3.31 to −0.68	0.003*

MDA=malondialdehyde; BMI=body mass index; CI=confidence interval; LDL=low density lipoprotein

Multiple R-squared: 0.159, adjusted R-squared: 0.1285

higher in the obese group than the non-obese group. Categorized by WCs, the result also showed the urinary MDA level in central obesity was significantly higher than the normal WC, consistent with previous studies that found serum MDA levels were significantly higher in the higher BMI group than the lower one[6,18-21]. Results showed a closed correlation between BMI and the end-product of polyunsaturated fatty acid. Studies have found higher level of oxidative stress levels in obese people, explained by mechanisms such as low-grade chronic systemic inflammation, hyperglycemia, or impairment of antioxidant defense systems[6,10].

Multivariate regression analysis showed that BMI, cholesterol, and LDL-cholesterol level related to urinary MDA. It was indicated that obese patients revealed a positive correlation with urinary MDA. Previous studies[21-24] showed strong correspondence between MDA and obese status, which is similar to the present research. The study of Venka et al in 2011 showed a positive correlation between MDA and cholesterol[25], which is consistent with the authors' research.

The researchers expected that LDL-cholesterol would have a positive correlation with MDA level. However, the present study results showed a trend of negative correlation, which is incompatible with the previous study[21]. In this case, the lowering level of urine MDA in high LDL-cholesterol patients could be explained by the use of statin, HMG-CoA reductase inhibitor, which has some evidence that it can significantly reduce systemic MDA concentration[26].

The present study has limitations, including that the references were performed using serum MDA, while the present study used urinary MDA. Therefore, the researchers had insufficient data to compare urinary MDA and serum MDA results. However, urinary collection is a non-invasive procedure compared to plasma collection for analyzing MDA or biomarkers. The test for urinary MDA should be further researched for validity and accuracy. The present study also used a questionnaire to report past behavior such as exercise, so recall bias should be a concern due to the subjects' memory inaccuracy. Finally, the present study was designed as a cross-sectional design and could not show direct causality.

Conclusion

Urinary MDA was significantly higher in the obese and central obesity group than the lower or normal BMI, WC group. Obesity could be an essential factor promoting the body's oxidative stress. Since oxidative stress is well known to be involved in the causes of lifestyle-related or chronic disease, clinician may soon use this specific biomarker to help detect and monitor the development of these diseases.

What is already known on this topic?

Oxidative stress implies a disproportion between oxidants and antioxidants, which can cause tissue injury. In addition, it negatively affected tissue and cellular structures such as DNA, protein, lipids, lipoproteins, and cell membranes. MDA is an end-product of polyunsaturated fatty acid peroxidation. Therefore, it is comprehensively used as a biomarker or a reliable tool for assessing oxidative stress in the whole body. The authors intend to determine the association between urinary MDA, the biomarker of oxidative stress, and obesity status.

What this study adds?

The urinary MDA levels are significantly higher in the obese group than in the non-obese group. Categorized by WCs, the result also showed the

urinary MDA level in central obesity is significantly higher than normal WC.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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