Original article

Increased Serum Selenium Levels and Metabolic Factors are Associated with Alterations in Biomarkers of Glucose Metabolism

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ABSTRACT

Selenium is one of the trace elements that has been reported in the pathogenesis of T2DM through oxidative stress and inflammatory pathways. Therefore, the objectives of the present study were to determine serum selenium levels and other factors affecting serum selenium levels and to analyze the association between serum selenium levels, fasting blood glucose (FBG), HbA1c and risk factor develop to T2 DM. Total participants of 890 cases (aged 50-75 years) from a cohort study conducted in 2013 with general characteristic and biochemical data. Serum selenium was measured by ICP-MS. The results showed no significant effects of smoking cigarette, alcohol consumption and dietary pattern on serum selenium levels. Statistical analysis by three groups of subjects with criteria of T2DM by HbA1c level was performed, as normal HbA1c group (HbA1c < 5.7% with subgroups of without and with metabolic components) and T2DM risk group (HbA1c $\geq$ 5.7%). The means of serum selenium levels in normal HbA1c group, without and with metabolic components (124.3±14.3 µg/L and 129.1±17.6 µg/L, respectively) were lower than those in risk group (132.2±17.1 µg/L). There were significant differences among all three groups in mean levels of clinical biochemical variables, including SBP, DBP, triglyceride total cholesterol and LDL-cholesterol. Moreover, subjects in the third serum selenium quartile had an increased risk of T2DM (increased levels of FBG and HbA1c) compared with those in the first quartile. In summary, dietary selenium supplements in general population and/or patients together with underlying molecular mechanisms in the disease pathogenesis related to selenium should be further studied

Keywords: Serum selenium, T2DM, HbA1c

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นิพนธ์ต้นฉบับ

การเพิ่มขึ้นของระดับซีลีเนียมในเลือดและปัจจัยด้านเมแทบอลิค มีความสัมพันธ์กับการเปลี่ยนแปลงของตัวชี้วัดทางชีวภาพของ Glucose metabolism

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บทคัดย่อ

ซีลีเนียม เป็นหนึ่งในกลุ่มแร่ธาตุปริมาณเล็กน้อยที่มีความจำเป็นต่อร่างกาย และมีรายงานความสัมพันธ์กับพยาธิสภาพของการเกิดโรคเบาหวานชนิดที่ 2 โดยเกี่ยวข้องกับกลไกการเกิดภาวะเครียดออกซิเดชันและการอักเสบ ดังนั้นวัตถุประสงค์ของการศึกษาครั้งนี้คือเพื่อศึกษาระดับซีลิเนียมในเลือด และปัจจัยต่างๆที่มีผลต่อระดับซีลิเนียมในเลือด รวมถึงศึกษาความสัมพันธ์ระหว่างซิลิเนียมในเลือดกับ fasting blood glucose (FBG), และ HbA1c ในเลือดที่มีผลต่อภาวะเสี่ยงต่อการเกิดเบาหวาน ในกลุ่มศึกษาจำนวน 890 คน (อายุ 50-75 ปี) จากโครงการวิจัยระยะยาวที่เก็บข้อมูลทั่วไปและผลทางชีวเคมีในปี คศ. 2013 การวัดระดับของซีลีเนียมในเลือดโดยใช้เครื่องมือ ICP-MS ผลการศึกษาพบว่าปัจจัยเรื่องการสูบบุหรี่ การดื่มแอลกอฮอล์และรูปแบบการบริโภคอาหารไม่มีผลต้อระดับซิลีเนียมในเลือด การวิเคราะห์ทางสถิติโดยแบ่งเป็น 3 กลุ่มโดยใช้ระดับของ HbA1c พบว่ากลุ่มที่มีระดับ HbA1c ปกติ (<5.7%) ที่มีและไม่มีองค์ประกอบของกลุ่มอาการเมตาบอลิค มีค่าเฉลี่ยระดับของซีลีเนียมในเลือด (124.3±14.3 µg/L และ 129.1±17.6 µg/L, ตามลำดับ) ต่ำกว่ากลุ่มเสี่ยงต่อการเกิดโรคเบาหวานชนิดที่ 2 ที่มีระดับ HbA1c ≥ 5.7% (132.2±17.1 µg/L) อย่างมีนัยสำคัญทางสถิติ นอกจากนี้พบว่าค่าเฉลี่ยของ SBP, DBP, triglyceride, total cholesterol และ LDL-cholesterol ของทั้ง 3 กลุ่มมีความแตกต่างกันอย่างมีนัยสำคัญ นอกจากนี้กลุ่มศึกษาที่มีระดับซีลิเนียมในเลือดอยู่ใน third tertile มีความเสี่ยงต่อการเกิดโรคเบาหวานชนิดที่ 2 (ประเมินจากการเพิ่มขึ้นของระดับ FBG และ HbA1c) มากกว่ากลุ่มที่อยู่ใน first tertile โดยสรุปจากงานวิจัยนี้พบว่าระดับซิลีเนียมในเลือดมีความสัมพันธ์กับความเสี่ยงต่อการเกิดโรคเบาหวาน การให้อาหารเสริมที่มีซิลีเนียมในกลุ่มคนทั่วไปและ/หรือกลุ่มเสี่ยงต่อโรคทางเมตาบอลิค รวมถึงกลไกระดับโมเลกุลที่เกี่ยวข้องกับซิลีเนียมและพยาธิสภาพของโรคดังกล่าว ควรจะได้รับการศึกษาต่อไป

คำสำคัญ: ระดับซีลีเนียมในเลือด โรคเบาหวานชนิดที่ 2 ระดับ HbA1c

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Introduction

Fundamentally, the antioxidant defense system is responsible for ameliorating deleterious effects of reactive oxygen species (ROS) in various cells and this system includes enzymatic and non-enzymatic antioxidants (i.e. glutathione, vitamin A, vitamin E and vitamin C). The enzymatic antioxidants are superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione reductase (GR), glutathione transferase (GST), and thioredoxinreductase. These enzymes require essential trace elements such as zinc, copper and selenium as co-enzymes. Moreover, trace element status in individual can influence on effective functions of antioxidant enzymes, including maintaining free radical homeostasis and also defensing against oxidative stress (1). Based on mechanisms of imbalance between ROS and antioxidant system, they can link to the pathogenesis of cardiovascular disease, atherosclerosis, hypertension, ischemia/reperfusion injury, diabetes mellitus, and neurodegenerative diseases. Therefore, role of antioxidants in the maintenance of cellular or redox homeostasis and of abnormal ROS-induced signaling pathways are very important. Current issues are also reviewed such as the question whether excessive formation of free radicals caused a downstream consequence of various disease and what are ameliorating effects of nutrients for decline oxidative stress.

Selenium (Se) is a well-known essential trace element found in a variety of foods including organ meat, seafood, dairy products, cereals, onion, garlic, mushroom and Brazil nuts. The content of selenium in foods depends on the concentration of selenium in soil or feed varied in different geographical area. This trace element is a basic component of selenoproteins and selenoenzymes which both of them regulate various cellular responses related to redox signaling, oxidative stress and protein folding (2). The recommended intake of selenium reported from US Recommended Dietary Allowance (RDA) in adult is average of 55 μg/day, whereas World Health Organization (WHO) has defined the maximum daily intake of selenium should not exceed 70 μg/day (3).

Various factors can influence on selenium status, including individual genetic variations, gender, age, smoking cigarette, alcohol consumption, some medications and underlying diseases of each person. For biochemical tests, determination of blood selenium concentration is generally considered as a useful biomarker for both selenium status and intake. However, selenium levels in other tissues such as hair and nails are also determined. Measurement of selenium in different biological samples has been reported with clinical implications. In case of plasma or serum selenium, this level can represent short-term status. Others such as erythrocyte, hair or toenails have often used as a measure of long-term selenium status. Mostly in certain populations, a large number of researches focused on the determination of total selenium level in serum. Furthermore, it is often difficult to compare results from different laboratories because of variations in methodology.

Evidences from the animal studies have shown risk of prolonged high selenium intake in potentiating insulin resistance and T2DM (4). Moreover, previous study in the

Asian population reported that an increased dietary selenium intake was associated with an increased risk of T2DM (5). However, additional researches are needed to explain diabetogenic effect related to high selenium intake. A systematic review showed that serum selenium levels were positively associated with T2DM in a J-curved style with relatively both low (<97.5 μg/l) and high serum selenium levels (>132.5 μg/l) (6). Several studies reported that patients with T2DM had higher serum selenium concentration compared with healthy subjects, and serum selenium levels had positively correlated with plasma glucose in T2DM (7-10). On the other hand, there were some clinical evidences demonstrated that patients with T2DM had significantly lower serum selenium concentrations compared with healthy subjects (11-14). Based on overall published researches, the links between serum selenium levels and T2DM are still controversial. Therefore, the objectives of the present study were to determine serum selenium levels and other factors affecting serum selenium levels and to analyze the association between serum selenium levels, fasting blood glucose (FBG), hemoglobin A1C (HbA1c) and risk factor develop to T2DM.

Materials and methods

Study population

Subjects were recruited from the Electricity Generating Authority of Thailand (EGAT) which was a cohort study conducted in 2013, aged 50-75 years. Total participants of 890 cases were enrolled in this study and they had completed the demographic characteristics, socioeconomic status, and physical examination. Weight in kilograms (kg) and height in centimetres (cm) were measured for calculation of the body mass index (BMI). Waist circumference was measured using a soft tape measure at the mid-point between the upper border of the iliac crest and the inferior margin of the last rib. Blood pressure was measured with the subject in the sitting position after a ten minute rest; two readings were taken with a minimum interval of ten minutes and the mean of the two readings was used to record the blood pressure. Exclusion criteria included 1) the abnormality of liver or renal function, which may disturb selenium metabolism; 2) the diagnosis with diabetes (followed by American Diabetes Association (ADA), 2016 (15) and who used antihyperglycemic drugs; 3) supplementation with selenium alone or multivitamins and 4) incomplete data related to all outcomes. This study was approved by the Ethic Committee on Human Right Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA2017/162).

Data collection

Venous blood sampling (10 mL) was performed after an overnight fast for biochemical analysis. These tests included blood glucose, hemoglobin A1C (HbA1c), total cholesterol (TC), triglycerides (TG), LDL-cholesterol, HDL-cholesterol, liver and kidney function test by auto-analyzer (Roche Cobas C6000). Serum selenium level was analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Agilent Technologies 7700x).

Statistical analysis

The demographic and clinical characteristics of participants were summarized using means ± standard deviation (SD) for

continuous variables and as proportions (%) for categorical variables. Independent T-test was used to evaluate differences of mean values between two groups. To compare differences in continuous demographic and clinical variables among three study groups, one way analysis of variance (one-way ANOVA) were applied. Two-sided *p value* < 0.05 indicated statistical significance. All analyses were done with SPSS software (version 15; SPSS Inc, USA).

Results

General characteristics of study population, classified by gender are shown in Table 1. There were 611 of male (68.8%) and 279 of female (31.2%) with average age 60.6±4.3 and 59.6±3.9 years, respectively. The means of age, BMI, systolic and diastolic blood pressures among all subjects were 60.3±4.2 years (range 53-72 year), 25.00±3.53 kg/m^2 (range 18.5- 45.4 kg/m^2), 130.50±16.92 mmHg (range 86-207 mmHg) and 79.53±10.67 mmHg (range 45-127 mmHg), respectively. Significant means of SBP and DBP in men were greater than women (132.5 vs 125.9 mmHg for SBP and 81.1 vs 76.1 mmHg for DBP ($p<0.001$). For smoking status, 51.1% male were smokers, together with alcohol drinkers as 78.9 % in males and 29.0% in females.

Table 1. General characteristics of the study population

Characteristics	Total	Gender	
		Males	Females
N (%)	890 (100)	611 (68.8)	279 (31.2)
Menopause, n (%)	221	-	221 (24.8)
Age (years ±SD)	60.36±4.27 (53-72)	60.69±4.36 (53-72)	59.63±3.99 (53-72)
Body mass index (kg/m^2)	25.00±3.53 (18.5-45.4)	25.09±3.37 (18.6-38.7)	24.80±3.87 (18.5-45.4)
Systolic blood pressure (mmHg)	130.50±16.92 (86-207)	132.56±16.14 (94-207)	125.98±17.71[a] (86-186)
Diastolic blood pressure (mmHg)	79.53±10.67 (45-127)	81.08±10.29 (57-127)	76.14±10.72[a] (45-127)
Smoking status, n (%)			
● Nonsmokers	572 (64.3)	299 (48.9)	273 (97.8)
● Smokers	318 (35.7)	312 (51.1)	6 (2.2)
Alcohol consumption, n (%)			
● Nondrinkers	327 (36.7)	129 (21.1)	198 (71.0)
● Drinkers	563 (63.3)	482 (78.9)	81 (29.0)
Educational levels			
● <12 years (%)	372 (41.8)	277 (45.3)	95 (34.1)
● ≥12 years (%)	518 (58.2)	334 (54.7)	184 (65.9)

[a] Significant difference from male with $p<0.05$

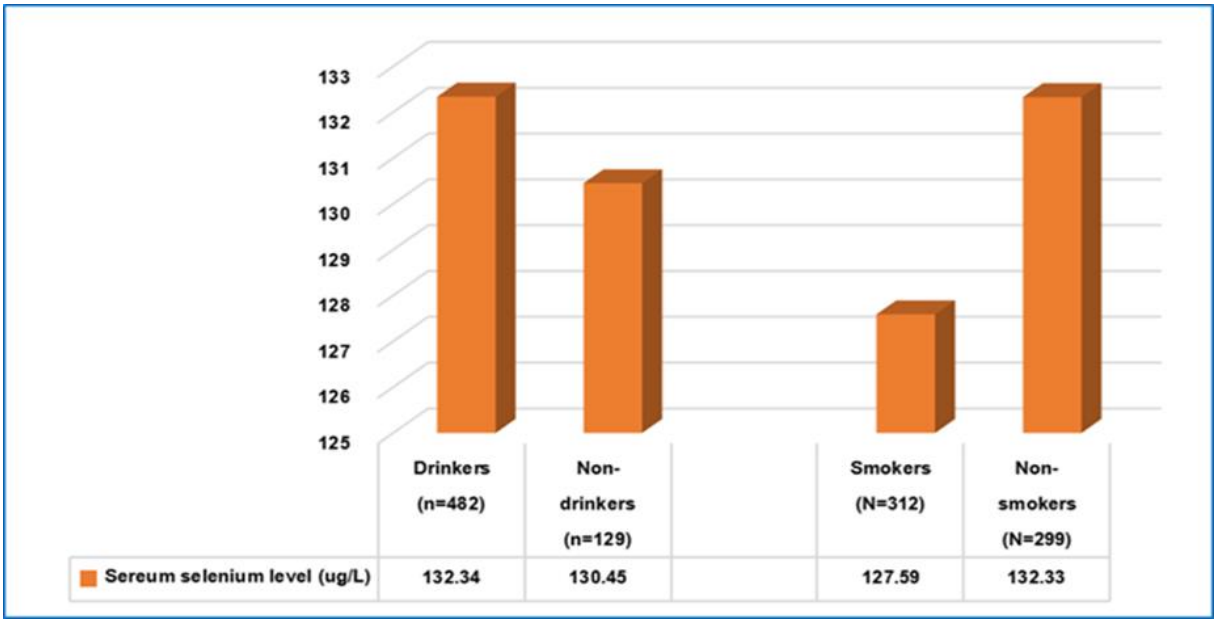

Figure 1. Serum selenium levels in male subjects (n=611) classified by alcohol intake and cigarette smoking

Biochemical assessment for metabolic syndrome was investigated according to Adult Treatment Panel III-2001 (ATP III) criteria (Table 2). Average FBG and HbA1c levels in male subjects were higher than female (99.96±22.75 mg/dL vs 93.00±15.85 mg/dL, p <0.001 and 5.71±0.84% vs 5.60±0.55%, p <0.05). For diagnosis of T2DM, 32.2 % with pre-diabetes (HbA1c 5.7-6.4%) and 9.6% with diabetes (HbA1c > 6.4%) were observed this study population. Triglyceride level in male subjects (139.01±67.2 mg/dL) was statistically greater than female (108.5±53.4 mg/dL, p<0.001). Means of total cholesterol and LDL-cholesterol in all subjects and both gender were above reference levels for metabolic syndrome criteria. Mean levels of albumin, liver enzymes and kidney function test were in reference values. Kidney function is partly represented by serum BUN and creatinine.

Mean serum selenium level for all study subjects was 130.2±17.5 µg/L, with significantly higher level in male than female (131.5±17.2 µg/L vs 127.3±17.7 µg/L, p<0.001). The results have been analyzed in relation to smoking and alcohol consumption habits, only in male subjects (as shown in Figure 1). The mean concentration of serum selenium for the group of smokers has been found to be lower compared to the group that does not smoke. However, the difference is without statistical significance. There were no differences in serum selenium levels between the drinking and non-drinking groups (132.34 vs 130.45 µg/L, p> 0.05). Diet is a factor that possibly effect on serum selenium levels. Frequency of food consumption in total population such as red meats, poultry, fresh water and sea fish, seafood, egg were included for data analysis. Consumption frequencies were classified by at least 1 time/day, at least 1 time/week, at least 1 time/month and never or at least 1 time/month. The result showed that there were no significant differences in serum selenium levels with various food items (Table 3).

Table 2. The biochemical profiles related to risk of T2DM classified by gender

Variables	Total	Gender		Reference
		Male	Female	value
Fasting blood glucose (mg/dL)	97.78±21.07 (71-251)	99.96±22.75 (71-251)	93.00±15.85[a] (71-212)	<100
HbA1c (%)	5.67±0.76 (4.10-11.10)	5.71±0.84 (4.10-11.10)	5.60±0.55 [a] (4.40-9.60)	<5.7
Triglyceride, (mg/dL)	129.44±64.74 (37-385)	139.01±67.20 (37-385)	108.50±53.41 [a] (39-373)	<150
Total cholesterol, (mg/dL)	211.49±37.44 (85-298)	207.49±38.14 (85-296)	220.24±34.33 [a] (130-298)	<200
LDL-cholesterol (mg/dL)	140.11±34.67 (37-244)	139.35±36.12 (37-244)	141.79±31.24 (65-226)	<130
HDL-cholesterol (mg/dL)	58.79±15.07 (28-112)	54.58±13.02 (28-109)	68.02±15.16 [a] (37-112)	>40
Albumin (mg/dL)	4.76±0.23 (3.91-5.53)	4.80±0.23 (3.91-5.53)	4.70±0.23 [a] (3.94-5.35)	3.5-5.5
ALT (U/L)	26.27±14.42 (4-103)	28.05±14.50 (4-102)	22.37±13.49 [a] (5-103)	0-55
AST (U/L)	24.15±8.07 (11-70)	24.53±7.98 (11-69)	23.30±8.21 [a] (13-70)	5-34
Alkaline phosphatase (U/L)	70.38±17.99 (34-190)	68.64±16.68 (34-144)	74.01±20.01 [a] (34-190)	40-150
Blood urea nitrogen (mg/dL)	12.50±2.86 (4.60-25.30)	12.77±2.77 (4.6-25.30)	11.89±2.96 [a] (5.20-24.10)	9-20
Creatinine (mg/dL)	0.95±0.17 (0.50-1.31)	1.04±0.11 (0.71-1.31)	0.76±0.11 [a] (0.50-1.03)	0.73-1.18
Serum selenium (µg/L)	130.25±17.53 (80.10-266)	131.57±17.27 (80.10-266)	127.36±17.77 [a] (96.95-241.84)	80-140

Note: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), [a] Significant difference from male with $p<0.05$

Table 3. Mean of serum selenium levels and contribution to participant's intake from different food groups

Parameters	At least 1 time/day	At least 1 times/week	At least 1 time/month	Never or <1 times/month
Red meat	130.20±17.96 (n=192)	130.47±17.24 (n=578)	128.96±18.12 (n=44)	129.43±18.56 (n=76)
Poultry	131.54±15.56 (n=81)	130.56±17.91 (n=584)	127.45±15.22 (n=99)	130.17±18.56 (n=126)
Fresh water and sea fish	131.54±17.68 (n=138)	130.81±17.85 (n=592)	126.84±15.20 (n=72)	127.23±16.43 (n=88)
Seafood	130.73±18.28 (n=37)	131.54±18.19 (n=396)	129.48±15.22 (n=223)	128.71±18.25 (n=234)
Egg	129.74±15.59 (n=159)	130.92±18.13 (n=649)	123.54±15.13 (n=20)	126.66±15.82 (n=62)

Further statistical analysis by three groups of subjects with criteria of T2DM by HbA1c level was performed, as normal HbA1c group (HbA1c <5.7% with subgroups of without and with metabolic components) and T2DM risk group (HbA1c ≥5.7%) (Table 4). The means of serum selenium levels in normal HbA1c group, without and with metabolic components (125.4±18.5 µg/L and 129.1±17.6 µg/L, respectively) were lower than those in risk group (132.2±17.1 µg/L). There were significant differences among all three groups (normal HbA1c group without and with metabolic components and T2DM risk group) in mean levels of clinical biochemical variables ($p < 0.05$) such as SBP, DBP, triglyceride, total cholesterol and LDL-cholesterol. For FBG, HbA1c and HDL-cholesterol levels in T2DM risk group were significantly different from both groups of normal HbA1c group without and with metabolic components.

In this study, tertiles of serum selenium were performed to elucidate the potential effects of this trace element on glucose metabolism through changes in fasting blood glucose and HbA1c, as shown in Table 5. The mean and range values for each tertile of serum selenium were 113.9 µg/L (80.1-122.7) for T1, 128.4 µg/L (122.9-134.5) for T2, and 148.2 µg/L (134.7-266.0) for T3. The increased serum selenium levels were found with increased both risk markers for T2DM as seen by higher FBG in subjects with selenium T3 (148.2±17.5 mg/dL) than those with T2 (128.4±8.9 mg/dL) and T1 (113.9 ±5.2 mg/dL) ($p<0.05$).

Table 4. Clinical and biochemical parameters related to metabolic components in normal HbA1c and T2DM risk groups

Variables	Normal HbA1c group (HbA1c <5.7%)		T2DM Risk group HbA1c ≥5.7%)
	Without metabolic components	With metabolic components	
N (%)	33 (3.7)	485 (54.5)	372 (41.8)
Body mass index (kg/m^2)	21.01±1.45 (18.5-22.8)	24.36±3.17 (18.5-37.7)	26.19±3.63 [a, b] (18.5-45.4)
Systolic blood pressure (mmHg)	115.67±10.42 (90-130)	129.44±16.35[a] (86-182)	133.19±17.30 [a, b] (90-207)
Diastolic blood pressure (mmHg)	70.67±7.21 (49-82)	79.57±10.36[a] (45-115)	80.26±10.96 [a] (54-127)
Fasting blood glucose (mg/dL)	86.42±6.93 (74-100)	89.80±8.43 (71-121)	109.18±27.24 [a,b] (73-251)
HbA1c (%)	5.23±0.21 (4.8-5.6)	5.26±0.28 (4.10-5.60)	6.25±0.85 [a,b] (5.70-11.10)
Triglyceride (mg/dL)	83.42±28.33 (43-150)	121.53±59.93 [a] (37-384)	143.84±69.36 [a,b] (44-385)
Total cholesterol (mg/dL)	176.73±23.49 (85-199)	216.15±32.42 [a] (118-269)	208.49±42.38 [a,b] (108-298)
LDL cholesterol (mg/dL)	105.03±18.24 (37-129)	143.82±30.18 [a] (60-199)	138.39±39.14[a] (45-244)
HDL cholesterol (mg/dL)	64.24±14.86 (33-91)	60.50±15.46 (28-112)	56.09±14.13[b] (28-109)
Serum selenium levels (µg/L)	124.36±14.32 (92.34-180.93)	129.05±17.63 (80.10-253.59)	132.23±17.12 [a,b] (95.18-266)

[a] Significant difference from normal HbA1c without metabolic components, $p<0.05$

[b] Significant difference from normal HbA1c with metabolic components, $p<0.05$

Table 5. Selenium tertiles and their relations with biomarkers and various risk factors of T2DM

Risk factors of T2DM	Selenium level (µg/L)		
	Tertile 1 (N=296)	Tertile 2 (N=297)	Tertile 3 (N=297)
Serum selenium levels (µg/L)	113.9±7.1 (80.1-122.7)	128.4±3.33 (122.9-134.3)	148.2±16.4 (134.7-266.0)
Body mass index (kg/m^2)	25.2±3.8 (18.5-45.4)	24.9±3.4 (18.5-38.7)	24.8±3.3 (18.5-37.7)
Systolic blood pressure (mmHg)	131.5±17.5 (85.5-200.5)	130.2±15.7 (85.5-205.5)	132.6±16.6 (91.5-184.0)
Diastolic blood pressure (mmHg)	76.3±10.7 (47.0-118.5)	78.6±9.5 (53.0-130.0)	84.2±10.9 [a,b] (52.0-119.0)
Fasting blood glucose (mg/dL)	113.9±5.23 (71-159)	128.4±8.95[a] (85-196)	148.2±17.52 [a,b] (95-251)
HbA1c (%)	5.57±0.87 (4.10-9.65)	5.68±0.43 (4.56-9.84)	5.94±0.98[a] (5.63-9.60)
Triglyceride (mg/dL)	118.4±59.3 (37-379)	127.3±60.4 [a] (45-385)	141.9±71.7 [a,b] (43-384)
Total cholesterol (mg/dL)	207.2±38.0 (85-293)	210±36.7 (117-294)	219.9±38.6[a] (112-298)
LDL-cholesterol (mg/dL)	134.8±35.1 (37-244)	140.2±33.9 (55-213)	142.2±34.8[a] (60-229)
HDL-cholesterol (mg/dL)	59.2±14.6 (28-112)	58.1±14.5 (28-112)	59.0±16.0 (29-110)

[a, b] significant different from selenium tertile 2 and 3 at $p<0.05$, respectively.

Moreover, there were significant differences in HbA1c levels between subjects with selenium T3 and those with selenium T1 (5.94±0.8 mg/dL vs 5.57±0.9md/dL, $p<0.05$). No significant association was found between selenium tertile and some parameters such as BMI, SBP and HDL-cholesterol levels. Regarding hypertension, DBP increased significantly across selenium tertiles. Subjects with serum selenium in T3 (84.2±10.9 mmHg) have higher DBP than those with T1 (76.3 ±10.7 mmHg) and T2 (78.6±9.5 mmHg) ($p<0.05$). While triglyceride, total and LDL-cholesterol were statistically associated with increased serum selenium. A potential interactive role of high selenium level in the interphase of diabetogenesis lead to the additional analysis in the present study. Data only subjects with the highest tertile of selenium (T3) were divided into three groups (group 1, 2

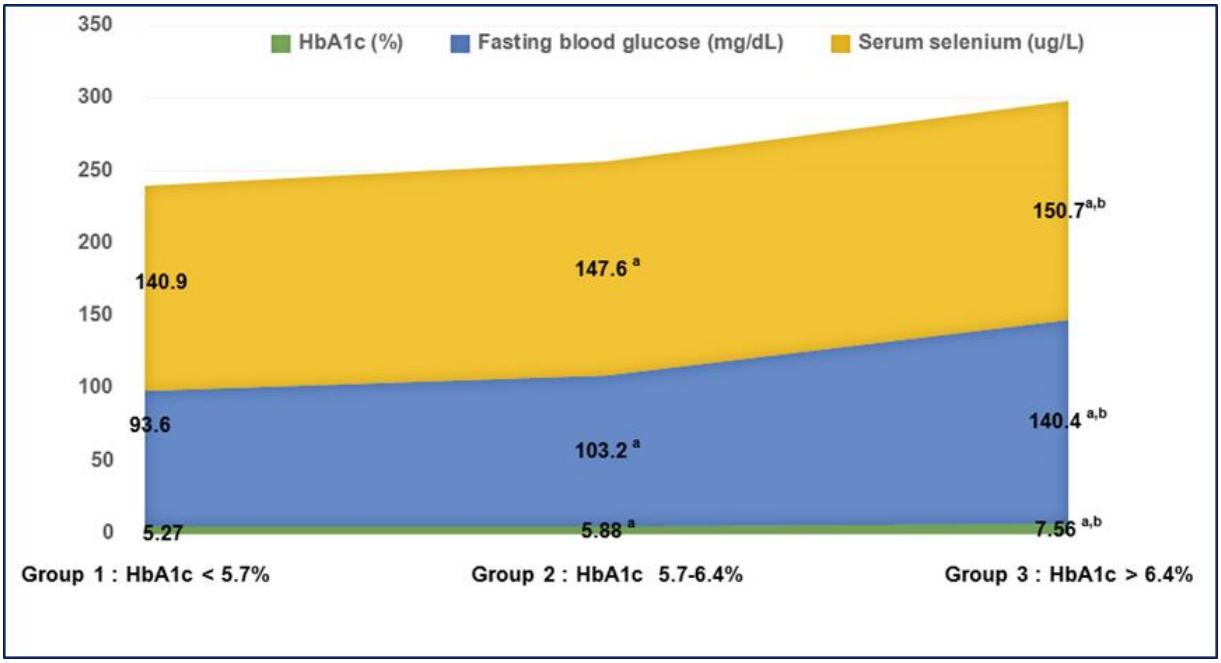

Figure 2. Association between serum selenium in the 3rd tertile, fasting blood glucose and HbA1c levels among three groups of HbA1c status ([a,b]significant different from HbA1c levels <5.7 and 5.7-6.4, $p<0.05$, respectively).

and 3) based on HbA1c levels, as demonstrated in Figure 2. Group 3 with the highest level of HbA1c showed the highest increased levels of serum selenium (150.7 μg/L) and fasting blood glucose (140.4 mg/dL) compare to group 1 (140.9 μg/L for serum selenium and 93.6 mg/dL for fasting blood glucose) and group 2 (147.6 μg/L for serum selenium and 103.2 mg/dL for fasting blood glucose) with $p < 0.05$.

Discussion

It is worthy of note that high generations of ROS and RNS can lead to oxidative stress which is one of the main mechanisms linking to T2DM (16). Based on ROS generation, they can trigger the important molecular mechanisms involved in hyperglycemia-induced oxidative tissue damage. Therefore, the inhibition or diminishment of intracellular free radical formation by antioxidant can provide a therapeutic strategy to prevent oxidative stress and diabetic vascular-induced complications. In the previous studies, the concept of selenium is an essential trace mineral known for its antioxidant function, leading to the expectation that selenium would play a protective role against diabetes mellitus. In addition, there was data supported that selenium acted as an insulin-mimic and antidiabetogenic parameters (17). However, animal experiments declared that overproduction of selenium-related compounds induced hyperglycemia in mice (18). Later, observational studies and randomized controlled trials suggested that suprasupplemented selenium and high serum selenium levels were probable risk factors for development of T2DM (19). These evidences suggested that the dual role of selenium was

triggered by selenoprotein P and glutathione peroxidase, regulating diabetes-associated hepatokine and ROS (4, 20). In Thai population, there was no large study that compared serum selenium levels and clinical markers of glucose metabolism between subjects with diabetes and non-diabetes. Therefore, the present study has mainly focused on the complex interaction of selenium and risk of T2DM in Thai population. Means of serum selenium in total population and both gender were 130.25±17.53 µg/L and 131.57±17.27 µg/L for males; 127.36±17.77 µg/L for females (Table 2). Data of serum selenium in Thai population are limited. Current study in Thais found that serum selenium level of surgical Intensive Care Unit (ICU) patient was below normal physiological range (70 to 150 µg/L) and healthy subjects was 106.95 µg/L (21). Previous study in Greece found that the mean concentration of total serum selenium was 91.8±33.7 µg/L (N=506). No statistically significant difference of selenium levels was observed between genders (90.5±35.1 µg/L for males and 93.9±31.6 µg/L for females, p=0.27) despite the worse health indices of males (smoking, BMI, blood pressure, levels of serum lipids, and inflammatory markers) (22).

Dietary pattern and frequency is well known as the important determinant of the serum selenium levels, especially the consumption of foods rich in selenium such as red meat, fish, and dairy products. Our result showed that there were no significant differences of serum selenium and frequency of food consumption (Table 3). Similar to study in Greek Adults, the dietary patterns of males and females did not affect significantly on the mean concentrations of serum selenium of the two groups (22). Whereas another study, dietary intake was assessed in 205 institutionalized elderly by means of a 1-year food frequency questionnaire (FFQ). Influences of smoking and alcohol consumption on the serum selenium level were not observed in this study (Figure 1). In contrast to another study, smokers appeared to be significantly lower of serum selenium concentration than those in non-smokers who have not been smoking for at least 10 years (23). However, there were some evidences that supported influence of cigarette smoking on antioxidant selenium level. Since cigarette smoke is a complex mixture of particulate matter and numerous toxicants with reactive properties, such as nitrogen oxides, organic peroxides and hydroperoxides, free radicals, and polycyclic aromatic hydrocarbons. Antioxidant, for example selenium may be important in detoxifying the oxidants inhaled in cigarette smoke (24). According to alcohol consumption in this male group, it did not influence the serum serenium concentration. Similar to previous study, serum selenium levels between drinker with consuming more alcohol and those with much alcohol showed not statistical difference (23). The mechanisms leading to the changes in blood selenium concentration in drinkers are inconclusive with some explanations such as inadequate dietary intake, increased losses /reduced hepatic storage capacity of selenium or liver dysfunction by alcohol consumption (24).

The American Diabetes Association (ADA) has been proposed that glycated haemoglobin (HbA1c) is an optional assay for diagnosing diabetes and also for detecting

individuals at increased risk of the disease (25). In addition, this marker can be used as a predictor for developments of diabetes onsets which is better than fasting blood glucose (FBG) for predicting chronic metabolic complications and cardiovascular risks. Therefore, this study has selected HbA1c to identify individuals at increased risk of diabetes. HbA1c between 5.7 and 6.4% (HbA1c 5.7–6.4%) is considered a category of increased risk for diabetes, refer to impaired fasting glucose (IFG) or pre-diabetes. Based on HbA1c levels in all subjects, the prevalence of pre-diabetes (HbA1c 5.7–6.4%) and diabetes (HbA1c ≥6.5%) were 32.2% and 9.6%. Previous study (2009) in Thai adults aged ≥35 years showed prevalence of diabetes and impaired fasting glucose levels were 9.6±0.7% and 5.4±0.6% (26). Current data in Thai National Health Examination Survey has been reported that the diabetes prevalence in Thailand has been increasing dramatically during the past decade, from 7.0% in 2004 to 9.7% in 2014 (27). However, the diabetes prevalence in the present study was lower than that in Malaysia (22.9% in 2013) and in Singapore (11.3% in 2010) (28, 29).

A wide variety of factors are identified with development of T2DM, such as age, gender physical inactivity, cigarette smoking, alcohol consumption and dietary pattern together with genetic predisposing factors and other metabolic components. In this study, some factors were investigated related to alterations in FBG, HbA1c and serum selenium levels (Table 1 and 2). Means of FBG and HbA1c in male group were significantly higher than those with female. The effect of gender on risk of T2DM in a manner of complex interaction between endocrine, social and genetic factors. To exemplify the association between metabolic factors and HbA1c level as a biomarker of glucose metabolism, three groups of subjects with metabolic outcomes were presented in Table 4. Moreover, the interaction between selenium and glucose homeostasis is one of the main focused outcomes of this study. Both sub-analysis of serum selenium in three tertiles and three groups of non-diabetes, prediabetes and diabetes were conducted with suggestive evidences. The increased serum selenium levels were found with increased both risk markers for T2DM as seen by higher fasting blood glucose in subjects with selenium T3 (148.2±17.5 mg/dL) than those with T2 (128.4±8.95 mg/dL) and T1 (113.9±5.23 mg/dL) ($p<0.05$). There was significant different in HbA1c level between subjects with selenium T3 and those with selenium T1 (5.94±0.98 mg/dL vs 5.57±0.87md/dL, $p<0.05$), as seen in Table 5. Moreover, the mean values for serum selenium in normal , pre-diabetes and diabetes group (based on HbA1c levels) were 140.9±11.9 µg/L, 147.6±12.6 µg/L and 150.7±21.8 µg/L, respectively with statistical differences between normal and diabetes group ($p<0.05$), as described in Figure 2. Study in Northern Taiwan as a hospital-based case–control study of 847 adults aged more than 40 years (diabetes: non-diabetes =1:2), the results were similar to this present study. In addition, study in U.S. population found that mean serum selenium was 137.1 µg/L. The multivariable adjusted odds ratio [95% confidence interval (CI)] for diabetes comparing the highest quartile

of serum selenium (> or = 147 µg/L) with the lowest (< 124 µg/L) was 7.64 (95% CI; 3.34-17.46). Further analysis by spline regression models, the prevalence of diabetes as well as glucose and glycosylated hemoglobin levels increased with increasing selenium concentrations up to 160 µg/L (8). Based on overall outcomes from this study, pivotal metabolic pathways involving selenium and diabetes could be proposed as a result of redox paradox of insulin signalling, a concept that refers to facilitated insulin action by insulin-stimulated ROS.

Conclusion

A large number of studies have investigated the association between blood selenium levels on T2DM, but the conclusions are controversial. Outcomes from the present study have partly supported a complex interaction of this trace element with glucose metabolism. Subjects in the highest serum selenium quartile had an increased risk of diabetes compared with those in the first quartile. These results are based on a population with adequate selenium status, and cannot be extrapolated to populations with low selenium intake and status. Micronutrients and underlying cellular and molecular mechanism in the pathogenesis of T2DM at different levels, including genetic predisposition and dietary factors appear to be involved and needed further investigation.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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