Original Article

The Relationship between Characteristics of Cigarette Smoking and Cytokine/Antioxidant Status in Thai Males

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Objective: To compare enzymatic and non-enzymatic antioxidant levels and serum cytokine profile between smoker and non-smoker groups and to investigate the correlations between cytokine and antioxidant status and various characteristics of smoking consumption in Thai males.

Materials and Methods: The present cross-sectional study enrolled 182 Thai males (100 smokers and 82 non-smokers). Each subject was tested for erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPX), vitamin A, vitamin C, vitamin E, interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).

Results: Smokers had significantly lower vitamin C, vitamin E, and SOD levels than non-smokers, whereas IL-6 levels were significantly higher in smokers than non-smokers. Vitamin C, vitamin E, and SOD levels was significantly negatively correlated with cigarette pack-years, duration of smoking, and numbers of cigarette per day, whereas IL-6 and TNF-α levels were significantly positively correlated among various characteristics of smoking consumption. After adjusting for potential covariates, the authors found decreased values of vitamin C and vitamin E whereas increased values of IL-6 were still significantly associated with smoking ($p<0.05$).

Conclusion: These findings suggest the alterative values of antioxidant and cytokine biomarkers related to cigarette smoking in Thai males where smokers may be more likely to have weakening of the antioxidant defense systems and the modifying immune response.

Keywords: Smoking, Antioxidant status, Cytokines, Thai

Received 4 Jun 2018 | Revised 1 Nov 2019 | Accepted 8 Nov 2019

J Med Assoc Thai 2020;103(1):38-45
Website: http://www.jmatonline.com

It is widely accepted that cigarette smoking is one of the major leading behavioral causes of premature but preventable morbidity and mortality. Cigarette smoke contains many toxic compounds harmful to the health. There were evidences showing that mainly free radicals such as nitric oxide radicals, singlet oxygen, and hydrogen peroxide, induced by cigarette smoke, can cause oxidative damage to the complex biomolecules in cells[1]. Moreover, cigarette smoke can alter host response including vascular function, neutrophil or monocyte activities, and adhesion molecules[2]. Although an oxidant and antioxidant imbalance and inflammation have been found to play a role in the pathogenesis of chronic pulmonary diseases and cardiovascular diseases[3], it is still controversial as to whether there are correlations between cytokine or antioxidant status and smoking characteristics[4-7]. In addition, information regarding serum cytokines and antioxidant status found in smokers is rather scarce, especially from Thailand and other developing countries. This information derived from Thai smokers should be more applicable for

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How to cite this article: Suriyaprom K, Sirikulchayanonta C, Tungtrongchitr R, Namjuntra P, Phraephan S, Sornwatana T. The Relationship between Characteristics of Cigarette Smoking and Cytokine/Antioxidant Status in Thai Males. J Med Assoc Thai 2020;103:38-45.

Thai society and regional countries and pave way to stimulate and reinforce the quit-smoking activities for Thai smokers, to improve the quality of life and reduce the risk of various diseases related to smoking. The authors conducted the present research by comparing enzymatic and non-enzymatic antioxidant levels and serum cytokine profile between smoker and non-smoker subjects, and investigated the correlations between cytokine and antioxidant status and various characteristics of smoking consumption.

Materials and Methods

Subjects

The study protocol was approved by the Ethics Committee of Rangsit University (RSEC 19/2559) in accordance with the Declaration of Helsinki, and all participants gave written informed consent. The present cross-sectional study enrolled 182 Thai men aged 24 to 62 years where 100 were smokers and 82 were non-smokers, from urban and suburban residential areas of Bangkok, Thailand. For appropriate sample size calculation of the present study, the authors considered type one (α) and type two errors (β) of 0.05 and 0.20 (power 80%), respectively, and plasma vitamin C levels as a key variable based on the study of Jain et al[8]. The authors conducted a physical examination and obtained a medical history and lifestyle pattern on all study subjects. The subjects were excluded if they suffered from major ailments such as lung, liver, kidney, inflammatory, gastrointestinal, diabetes mellitus, or cardiovascular diseases, or if they consumed antioxidant vitamin supplements, which were diagnosed by a physician. Smoking characteristics, such as age at onset of smoking, duration of smoking (years), and number of cigarettes smoked were recorded by questionnaire. The quantity of cigarettes smoked for the whole period of smoking or cigarette pack-years, was calculated as the duration of smoking (years) multiplied by the number of smoked cigarettes, divided by 20 cigarettes per pack.

Anthropometric measurements

Weight, height, waist circumference (WC), and hip circumference (HC) were measured for all subjects. Blood pressure (BP) was measured by a nurse after the subject rested in a sitting position for 5 to 10 minutes. A body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Laboratory measurements

Seven milliliters of venous blood were obtained from each subject in the morning between 7.00 to 8.00 a.m., after an overnight fast and checked for glucose, triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels using a DADE Dimension AR®. Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald formula [LDL-C = TC – HDL-C – (TG/5)]. Glutathione peroxidase (GPX) and superoxide dismutase (SOD) levels were measured by using a Randox test combination (Randox Laboratories Ltd., United Kingdom). Serum vitamin C (ascorbic acid) levels were measured following the method described by Liu et al (1982)[9]. Serum vitamin A (retinol) and vitamin E (alpha-tocopherol) were measured by reverse-phase high performance liquid chromatography (RP-HPLC) as described by Schweigert et al[10]. The levels of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were measured by using enzyme-linked immunosorbent assay (ELISA) kits (EMD Millipore Ltd., Canada). To further minimize analytical variation, the same technician performed all assays and single lots of reagents were used. The inter-assay coefficient and intra-assay coefficient of variation for each of the parameters were less than 10% and 5%, respectively.

Data analysis

Statistical analysis was performed using SPSS for Windows, version 11.5 (SPSS, Chicago, IL). Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (twenty-fifth percentile to seventy-fifth percentile) in the presence of abnormal distribution. For the comparisons between the smoker group and the non-smoker group, the independent samples t-test was used for the parameters with normal distribution and the Mann-Whitney U-test was used for the parameters with non-normal distribution. Spearman rank correlation was applied to calculate correlations among variables. The authors used logistic regression analysis to assess associations between smoking as a dependent variable and other potential factors. The results were considered statistically significant at a p-value less than 0.05. Goodness-of-fit of the logistic regression models was established by Hosmer-Lemeshow test.

Results

The present cross-sectional study enrolled 182 Thai males (100 smokers and 82 non-smokers) from suburban and urban Bangkok, Thailand. The age

at onset of smoking tended to be the teenage years and the average duration of cigarette smoking was nearly 10 years. Sixty-nine percent were smokers and 19.5% were non-smokers who lived with smoking family. Table 1 shows the distribution of smokers according to the quantity of cigarettes smoked (units in numbers of cigarette pack-years). Anthropometric and biochemical results of subjects with and without smoking are shown in Table 2. No significant differences in anthropometric and biochemical parameters were observed between these two groups, except for WC values. Antioxidant findings between the two groups are shown in Table 3. There were significant differences between smoker and non-smoker groups with regard to IL-6, vitamin C,

Table 1. Distribution of smokers according to cigarettes smoked for the whole period of smoking

Quantity of cigarettes smoked (pack-years)	n (%)
1 to 5	29 (29.0)
6 to 10	25 (25.0)
11 to 15	16 (16.0)
16 to 20	15 (15.0)
>20	15 (15.0)

Table 2. Age and anthropometric-biochemical measurements among smokers and non-smokers

Variables	Total subjects (n=182); mean±SD		p-value[a]
	Non-smokers (n=82)	Smokers (n=100)	
Age (years)	41.9±9.4	40.5±8.5	0.120
Weight (kg)	69.7±10.1	67.9±11.3	0.246
BMI (kg/m^2)	24.5±3.2	24.0±3.4	0.304
WC (cm)	85.7±7.1	83.2±8.1	0.021*
HC (cm); median (IQR)	95.0 (90.0 to 99.0)	95.0 (90.0 to 98.0)	0.984[b]
Systolic BP (mmHg)	128.8±13.4	127.0±12.6	0.345
Diastolic BP (mmHg)	79.3±7.8	80.9±9.3	0.165
Glucose (mg/dL)	83.0±11.0	81.5±10.0	0.606
Total cholesterol (mg/dL)	218.0±35.0	215.0±35.0	0.583
Triglycerides (mg/dL)	148.0±58.0	147.0±57.0	0.941
HDL-C (mg/dL)	50.0±12.0	49.0±13.0	0.717
LDL-C (mg/dL)	128.0±26.0	126.0±29.0	0.660

BMI=body mass index; WC=waist circumference; HC=hip circumference; BP=blood pressure; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; SD=standard deviation; IQR=interquartile range

[a] According to independent sample t-test, [b] According to Mann-Whitney U test, * p<0.05 was considered significant

Table 3. Antioxidant levels and cytokine measurements among smokers and non-smokers

Variables	Total subjects (n=182); median (IQR)		p-value[a]
	Non-smokers (n=82)	Smokers (n=100)	
IL-1β (pg/mL)	1.7 (1.3 to 2.5)	1.8 (1.3 to 2.5)	0.733[b]
IL-6 (pg/mL)	4.1 (2.9 to 6.2)	4.8 (3.5 to 6.7)	0.009*
TNF-α (pg/mL)	3.5 (2.5 to 5.4)	4.1 (2.8 to 5.7)	0.060[b]
Vitamin A (µg/dL); mean±SD	63.7±16.1	61.3±16.9	0.332
Vitamin C (mg/dL)	16.9 (10.5 to 19.7)	14.2 (5.3 to 18.2)	0.018[b]*
Vitamin E (µg/dL); mean±SD	968.4±325.0	845.0±366.0	0.020*
GPX (U/g Hb); mean±SD	52.7±13.0	50.4±12.7	0.280
SOD (U/g Hb)	1,587.2 (1,313.0 to 2,038.0)	1,301.5 (1,200.0 to 1,807.0)	0.010[b]*

IL-1β=interleukin-1 beta; IL-6=interleukin-6; TNF-α=tumor necrosis factor-alpha; GPX=glutathione peroxidase; SOD=superoxide dismutase; SD=standard deviation; IQR=interquartile range

[a] According to independent sample t-test, [b] According to Mann-Whitney U test, * p<0.05 was considered significant

Table 4. Correlation coefficients for smoking characteristics and the studied variables among all study subjects

	Cigarette pack-years	Duration of smoking	Numbers of cigarette per day
Cigarette pack-years	1.000	0.952**	0.980**
Duration of smoking	0.952**	1.000	0.893**
Numbers of cigarette per day	0.980**	0.893**	1.000
IL-1β	0.079	0.090	0.056
IL-6	0.270**	0.210**	0.274**
TNF-α	0.187*	0.184*	0.155*
Vitamin A	–0.070	–0.111	–0.053
Vitamin C	–0.158*	–0.195**	–0.148*
Vitamin E	–0.213**	–0.204**	–0.186 *
GPX	–0.106	–0.113	–0.101
SOD	–0.359**	–0.302**	–0.331**

IL-1β=interleukin-1 beta; IL-6=interleukin-6; TNF-α=tumor necrosis factor-alpha; GPX=glutathione peroxidase; SOD=superoxide dismutase

* p<0.05 was considered significant by using Spearman rank correlation, ** p<0.01 was considered significant by using Spearman rank correlation

Table 5. Logistic regression analysis of biochemical variables associated with smoking (n=182)

Variables	B	S.E.	Adjusted OR[a] (95% CI)	p-value
Glucose (mg/dL)	–0.003	0.011	0.99 (0.98 to 1.02)	0.683
Total cholesterol (mg/dL)	0.001	0.003	1.00 (0.99 to 1.01)	0.633
Triglycerides (mg/dL)	0.000	0.003	1.00 (0.99 to 1.01)	0.973
HDL-C (mg/dL)	–0.004	0.012	0.99 (0.97 to 1.01)	0.718
LDL-C (mg/dL)	0.001	0.005	1.00 (0.99 to 1.02)	0.649
IL-1β (pg/mL)	0.069	0.100	1.07 (0.87 to 1.32)	0.499
IL-6 (pg/mL)	0.165	0.061	1.18 (1.05 to 1.33)	0.010*
TNF-α (pg/mL)	0.094	0.052	1.10 (0.99 to 1.22)	0.073
Vitamin A (µg/dL)	–0.011	0.009	0.99 (0.97 to 1.01)	0.222
Vitamin C (mg/dL)	–0.055	0.023	0.95 (0.91 to 0.99)	0.016*
Vitamin E (µg/dL)	–0.005	0.001	0.99 (0.98 to 0.99)	0.022*
GPX (U/g Hb)	–0.010	0.009	0.99 (0.96 to 1.01)	0.193
SOD (U/g Hb)	0.000	0.001	1.00 (0.99 to 1.00)	0.317

OR=odds ratio; CI=confidence interval; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; TNF-α=tumor necrosis factor-alpha; IL-1β=interleukin-1 beta; IL-6=interleukin-6; GPX=glutathione peroxidase; SOD=superoxide dismutase

[a] According to adjustment for the covariates age and alcohol drinking, * p<0.05 was considered significant

vitamin E, and SOD levels. Smokers had significantly lower vitamin C, vitamin E, and SOD levels than non-smokers (p<0.05). IL-6 levels were significantly higher in smokers than non-smokers (p<0.01). Spearman's rank correlation results for the smoking characteristics and the studied variables are shown in Table 4. The cigarette pack-years were significantly positively correlated with duration of smoking and numbers of cigarette (p<0.01). IL-6 and TNF-α levels were significantly positively correlated with cigarette pack-years, duration of smoking and numbers of cigarette (p<0.05). Vitamin C, vitamin E, and SOD levels were significantly negatively correlated with the smoking characteristics (p<0.05). To evaluate the associations of smoking with biochemical parameters, the authors performed logistic regression analysis. When smoking was used as a dependent variable, the results showed that decreased values of vitamin C and vitamin E whereas increased levels of IL-6 were still significantly associated with smoking (p<0.05), even after adjusting the variable to the covariates age and alcohol drinking status, as shown in Table 5.

The Hosmer and Lemeshow goodness-of-fit test was not statistically significant ($p>0.05$), meaning the fit between the predictive model and the data was acceptable.

Discussion

In Thailand, the male smoking prevalence is about 18.4 times, compared with the female prevalence in 2014[11]. To avoid the gender-influenced changes on the cytokine levels, females were not enrolled in the present study. The authors found significant differences in some studied enzyme and non-enzyme antioxidants and cytokines between Thai male smokers and non-smokers. Because cigarette smoke contains significant quantities of oxidative species in both the gas and tar phase, smoking increases reactive oxygen species (ROS) production, which is a significant source of oxidative-stress generation[1]. Zhang et al reported that the oxidative injury can induce production of inflammatory cytokines[12]; however, the results of smoking on cytokines have been inconsistent in different populations. When the effect of smoking is examined in the present study with regards to TNF-α and IL-1β levels, the present study showed no significant influences of smoking to these two inflammatory cytokines in Thais. The result on TNF-α level in the present study was in agreement with the observations in monozygotic twins[13] and Turkish population[14]. Moreover, some studies in Swedish[15] and Iranian population[16] reported no influence of smoking on IL-1β concentrations, which is in agreement with the present study in Thai males; whereas studies of smokers in Spain[17] and Italy[18] had a greater expression of TNF-α and IL-1β.

IL-6 is secreted by the immune cells, endothelial cells, and fibroblasts in response to environmental insults. It is a pleiotropic cytokine that has both proinflammatory and anti-inflammatory functions that affect processes ranging from immunity to tissue repair and metabolism[19]. The anti-inflammatory properties of IL-6 are illustrated by IL-6−/− mice, which exhibit liver inflammation and insulin resistance[20] and IL-6 classic signaling can mediate the apoptosis inhibition and the regeneration of intestinal epithelial cells[21]. Whereas IL-6 trans signaling also leads to recruitment of monocytes to the inflammation site. Additionally, a study of Emami et al[22] indicated that serum IL-6 level is a sensitive biomarker to predict inflammation. When the effect of smoking is examined with regards to IL-6 levels, the present study found the effect of cigarette smoking on increasing of IL-6 levels and this is in line with the previous studies in Italy[4], Iran[22] and the Framingham offspring study[23]. Furthermore, Pantano et al found that oxidative stress can induce the transcription factors nuclear factor kappa B (NFκB), a central role in the inflammatory response, leading to the transcription of genes encoding inflammatory cytokines[24] and one potential mechanism can be increase in oxidative stress by components of cigarette smoke especially ROS. de Moraes et al also confirmed that smoking cessation did not decrease IL-6 levels since increased IL-6 was mainly associated with smoking burden in chronic pulmonary disease patients[25]. Moreover, the IL-6 levels were slightly lower in females compared to males and smoking intensity was the main predictor of IL-6 high levels[26]. Therefore, the present study evaluated the effects of smoking on cytokines only in male to eliminate the reflection on gender bias to the cytokine levels. The authors' results supported that a dose-response relationship of pack-years of smoking, number of cigarettes smoked daily, and duration of smoking with the concentrations of cytokines including IL-6 and TNF-α. These relations appear to be a modifying immune response to toxins in smoking exposure and may lead to chronic diseases related to inflammation. On the other hand, previous studies in Chinese[7] and Iranian population[16] reported no influence of smoking on IL-6 levels. Therefore, the differences between the present study results and those of previous studies regarding the associations between smoking and cytokines may be the effects of gender, specimen collection, smoking behaviors, and different genetic backgrounds of the study populations to the cytokine concentrations. Tanaka et al speculated that racial variation of the gene polymorphism affects cytokine production, since there are such racial differences in allelic distribution[27]. Furthermore, the accurate measurement of cytokines in serum is complicated by the fact that IL-6 levels vary in a diurnal manner in the healthy individual, so the time of sampling would affect the final results[28]. Therefore, to minimize the outcome of the time in specimen collection, the authors collected venous blood samples from each subject in the morning between 7.00 to 8.00 a.m.

Inflammation is a manifestation of oxidative stress and the pathways that generate the mediators of inflammation are all induced by oxidative stress[29]. Cigarette smoke contains high concentrations of toxic gases and tiny particles that are rich of free radicals and non-radical oxidants that can initiate oxidative stress[1]. Protection against oxidative stress is provided by a system of antioxidants capable of neutralizing free radicals and preventing excess production of

ROS. In the present study, the authors investigated enzymatic and non-enzymatic antioxidant defense systems that are directly involved in the neutralization of ROS. The authors hypothesized that an alteration in antioxidant status may be reduced in smokers and the findings supports this hypothesis. The present study key findings in Thai population support the fact that smokers may be more susceptible to oxidative stress resulted in alterations of antioxidant defense systems such as SOD, vitamin C, and vitamin E levels. SOD catalytically converts the superoxide anion radical ($O_2^{\bullet-}$) into oxygen and H_2O_2 in presence of the metal ion cofactors such as copper (Cu) and zinc (Zn). The authors' observation of a significantly decreased SOD activity among Thai smokers compared to non-smokers was consistent with findings in India[8], Republic of Macedonia[5], and Egypt[30]. Moreover, previous study reported that cigarette smoke can induce a free radical overcharge that may be responsible for the inhibition of the gene expression of antioxidant enzymes including SOD that was counteracted by Vitamin E[31]. Uz et al also found a significantly depressed zinc status among heavy smokers compared to non-smokers[32] and decreasing cofactors including zinc may influence the activity of SOD. Therefore, the present study results suggest that increased ROS induced by smoking may result in the depletion or inactivation of SOD. In contrast to the present study results, Bolzán et al reported no changes of SOD activity in human blood and that gender also influenced SOD activity[33]. However, some studies reported significantly higher activity of SOD among smokers[34,35]. These studies suggest that increased SOD level is a protective defense mechanism to scavenge the excessive ROS produced by smoking[34].

Vitamin C and Vitamin E are potent antioxidant vitamins that can quench a variety of ROS, such as those found in environmental pollutants. Vitamin E functions primarily as a chain-breaking antioxidant that prevents propagation of lipid peroxidation. The oxidized vitamin E is then reduced by vitamin C, thereby effectively recycling this important lipid-soluble radical scavenger and limited oxidative damage to cell membrane[36]. The significantly decreased vitamin C and vitamin E concentrations related to smoking had still found in the present study, after adjusting the variable to the covariates age and alcohol drinking status. These findings in Thailand are similar to previous studies in India[8], Egypt[30], and Poland[37]. Jain et al[8] and Gomaa et al[30] found that plasma vitamin C was also significantly lower in cigarette smokers, and Szołtysek-Bołdys et al[37] found decreasing the concentration of plasma vitamin E, while not influencing significantly the plasma concentration of vitamin A. Moreover, previous finding also supported that smokers consume less milk, beans, fruits, and vegetables than non-smokers[8]. The authors' key findings in Thai population support the fact that smoking may be more susceptible to oxidative stress, resulted in weakening of the antioxidant defense systems, especially vitamin C and vitamin E, and this may reflect the protective defense mechanism to scavenge the ROS induced by smoking. This is in accord with vitamin C used in combination with vitamin E for reducing oxidative damage. Therefore, nutrition recommendations, especially a diet relatively high in antioxidant-rich foods is essential to Thai smokers for the maintenance of antioxidant status. In contrast to the present study results, smokers in local population of Pokhara[6] and in Western Nepal[38] did not show any significant difference from never-smokers with regard to vitamin E and vitamin C. However, the differences between the authors' results and those of previous studies regarding the associations between antioxidant vitamins and smoking may result from different dietary habits and smoking characteristics of study populations.

Limitations of the present study were that the study involved a relatively small number of subjects. Despite the relatively small number of subjects studied, the authors found consistent results in terms of smoking-induced alteration of antioxidant-cytokine biomarkers. The second was the smoking status was recorded by the self-report of the participants. Therefore, further studies should be considered regarding the smoking status by using serum cotinine assay and investigating the effects of gene-environment interactions especially in Thais.

In conclusion, the authors found that some antioxidants and cytokines were associated with smoking. Potential explanations of the present study findings for the increased cytokine concentrations and the decreased antioxidant biomarkers related to cigarette smoking in Thais may be due to the increased production of ROS, which resulted in the increased demand of antioxidant defense mechanisms and the modifying immune response.

What is already known on this topic?

There were studies about serum cytokine and antioxidant status among smokers in many countries, however, it is still controversial whether cytokines and antioxidants have some relationship with smoking.

What this study adds?

This study reveals that some antioxidant and cytokine biomarkers are related to cigarette smoking in Thai males. After adjusting for potential covariates, the result showed decreased values of vitamin C and vitamin E, and increased values of IL-6 that were still significantly associated with smoking. Vitamin C, vitamin E, SOD, IL-6, and TNF-α levels were significantly correlated with cigarette pack-years, duration of smoking, and numbers of cigarette per day.

Acknowledgement

The authors wish to express their sincere thanks to all volunteers and staff of the Faculty of Medical Technology, Rangsit University as well as staff of the Faculty of Tropical Medicine, Mahidol University, for their assistance in carrying out this research. The authors also thank the Center of Translation, Faculty of Liberal Arts, Rangsit University for proofreading this manuscript. The project was supported by funds from Rangsit University, Thailand.

Ethical consideration and conflicts of interest

The authors declare that we have no conflict of interest associated with this work and all ethical issues (such as plagiarism, double submission, etc.) have been considered carefully.

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