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Tumor necrosis factor alpha in smokers and non-smokers

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Abstract

Previous investigations have described a positive association between smoking and systemic inflammation. To compare serum Tumor necrosis factor alpha (TNF-α) between smoker and non-smoker men, fifteen healthy adult smoker and the same number non-smoker men matched for age and body mass index participated in this study by accessible sampling. To determine difference in serum TNF-α between two group, their blood samples were collected an overnight fast. Independent sample T-test was used to compare the serum levels of TNF-α between smoker and non smoker subjects. There was not significant difference in serum TNF-α between smoker and non-smoker groups. These data do not support the inflammatory property of smoking. Further studies are needed to clarify possible mechanisms by which smoking affect systemic inflammation by TNF-α or other cytokines.

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Introduction

This hypothesis has been proven many times that cytokine is influenced in response to a variety of diseases and are external stimuli, such as cigarette smoking. Recent sources identify TNF-α as the main chemical mediator of inflammatory responses in the face of negative bacterial infections and other infectious microbes and the spread of complications in acute infections. In fact, tumor necrosis factor is identified as TNF-α to make it distinct from TNF-β or lymphocyte toxin. Plasma or serum levels of this inflammatory cytokine have been reported higher in obese or insulin resistant individuals than normally weighed or healthy individuals (Ye, 2008). Although adipose tissue is the major source of secretion of this inflammatory cytokine it is also secreted by the macrophages and other cells. The role of TNF-α in inflammatory process has often been reported and has also been introduced as a synthesis stimulus of such interleukins as IL-8 (Gerszten *et al*., 1999). The significant and positive relationship between TNF-α and blood triglyceride levels, has been reported as a risk factor for cardiovascular diseases (Jovinge *et al*., 1998). This inflammatory cytokine has multiple functions such as cardiomyocyte hypertrophy and impaired contractile function (Murray *et al*., 2003). Blood circulatory levels of inflammatory cytokines in obese individuals are reported seven and half times greater than those of normal weight or underweight individuals (Kern *et al*., 1995). The stimulating role of TNF-α in increased production vLDL has been observed in some studies that describes the relationship of these cytokines with plasma TG (Qin *et al*., 2008).

Active inflammatory cells produce various inflammatory mediators in response to smoking, and inflammatory cytokines are the important of them. Importance of smoking in increasing inflammatory cytokines has been reported in some previous studies (Patrícia, 2011).

TNF-α is in fact, one of the most important cytokine secreted by adipose tissue increasing the presence of other inflammatory cytokine such as IL-6 and IL-1 in the blood (Weiss, 2005). The inhibitory effect of TNF-α inflammatory cytokines on the expression and serum levels of adiponectin has been reported in some literature (Miller *et al*., 2008). Changes in the levels of these inflammatory cytokines not only occur in the lungs and respiratory tract of smokers but also affect the levels of these cytokines in blood circulation (Dilyara *et al*., 2007). Some previous studies, report increased levels of TNF-α in smokers than in non-smokers (Tappia *et al*., 1995; Pessina *et al*., 1993). While some other studies report no difference between smokers and non-smokers in inflammatory cytokine (Keatings *et al*., 1996; McCrea *et al*., 1994). Recent studies have also shown that TNF-α plays an important role in developing smoking-related chronic obstructive pulmonary disease (COPD) (Churg *et al*., 2004; Churg *et al*., 2002) Given the contradictory previous findings in comparing this inflammatory cytokine in smokers and non-smokers, this study aims to compare the same in smoking and non-smoking athletic men.

Material and methods

Subjects

Fifteen healthy adult smoker and the same number non-smoker men were selected to participate in this study Groups were matched with respect to age and anthropometrical markers. This study was aimed to comparison serum TNF-α between smoker and non-smoker participants.

Inclusion and exclusion criteria

All participants were non-trained, so all subjects had participated in regular exercise for the preceding 6 months. A main inclusion criterion was at least 10 cigarettes smoking for 3 years. Subjects were reported to be not currently taking supplements of any kind, and having no major health problems (i.e., diabetes, cardiovascular disease, etc.).

After the nature of the study was explained in detail, informed consent was obtained from all participants. Subjects were asked to avoid doing any heavy physical

activity for 48 hours before blood sampling.

Anthropometrical measurements

After introduction and awareness of the subjects of the objectives of the study and once they had completed consent forms, all anthropometrical markers were measured in the morning following a 12-h fast in two group subjects. All anthropometric measurements were made by the same trained general physician and under the supervision of the same pediatrician. Anthropometric measurements of height, weight, percent body fat, and circumference measurements were taken in the physiology laboratory. Abdominal circumference and hip circumference were measured in the most condensed part using a non-elastic cloth meter. Waist to hip circumference ratio was measured by dividing the abdominal circumference into that of the hip. Body weight and height were measured with a standard physician's scale and a stadiometer, respectively when subjects were in a fasting state before the resting metabolism session. Body mass index was measured for each individual by division of body weight (kg) by height (m2).

Blood sampling and Assay

Blood samples were taken following an overnight 12-hour fast between 8:00 and 9:00 a.m. Subjects were asked to avoid doing any heavy physical activity for 48 hours before blood sampling. Blood samples were taken in order to measuring fasting serum TNF-α and comparison between two groups. Blood samples were dispensed into EDTA-coated tubes and centrifuged for 10 minutes in order to separate serum. Serum TNF-α was determined by ELISA method (Enzyme-linked Immunosorbent Assay for quantitative detection of human TNF-α). The Intra- assay coefficient of variation and sensitivity of the method were 6% and 5 pg/mL, respectively.

Statistical analysis

Statistical analysis was performed with the SPSS software version 15.0. The Kolmogorov-Smirnov test was applied to determine the variables with normal distribution. An Independent sample T-test was used to compare all variables between asthma and none-asthma subjects. P value of <0.05 was accepted as significant.

Results

In this study, serum TNF-α level was compared between adult smokers and non-smokers men. Table 1 shows the characteristics of anthropometrical and TNF-α level in two groups. Data were expressed as individual values or the mean ± SD. The values of physical and anthropometrical markers showed that studied subjects are overweight or obese. Data of independent method also showed no significant difference in serum TNF-α between smoker and none-smokers (p = 0.681). Of course, It should be noted that fasting serum TNF-a tended to (borderline) to be higher in smoker subjects than non-smokers.

Table 1. Characteristics of the study subjects according to fasting blood samples and anthropometrical measurements.

Variables	smoker	Non-smoker	ρ
Age (year)	*41.8 ± 4.8*	*42.8 ± 8.3*	*0.212*
Weight (kg)	*95.4 ± 4.5*	*93.8 ± 3.8*	*0.412*
Height (cm)	*174.9 ± 6.5*	*175.6 ± 6.7*	*0.312*
Body Fat (%)	*31.33 ± 3.4*	*30.94 ± 4.3*	*0.236*
Body mass index (kg/m²)	*31.51 ± 4.2*	*30.63 ± 3.3*	*0.325*
Abdominal circumference (cm)	*108.7 ± 7.6*	*106.3 ± 5.6*	*0.323*
TNF-α (pg/ml)	*38.33 ± 4.5*	*35 ± 11*	*0.681*

Values are means ± SD; BMI, body mass index; BF (%), Body fat percentage; WHO, Abdominal to hip circumference ratio.

Discussion

Despite this, most previous studies support higher levels TNF-α as an inflammatory cytokine in male smokers than non-smokers, but the statistical finding of t-test in this study showed no significant differences in baseline levels of these inflammatory cytokines in smoking and nonsmoking subjects. Indeed, regardless of at least 5 years of smoking in the smoker group, their TNF-α level, in smokers although not higher than in nonsmokers, were statistically insignificant. It is well established that smoking damages the pulmonary-respiratory immunity function and leads to increased release of inflammatory mediators and is ultimately associated with increased prevalence of lung disease. Recent studies have shown that TNF-α plays an important role in smoking-related chronic obstructive pulmonary disease COPD and continuation of respiratory routes inflammation (Chung *et al.*, 2001). Moreover, experimental studies on animals have shown that tobacco consumption increases levels of TNF-α in both serum and lung alveolar fluid (Pessina *et al.*, 1993; Pang *et al.*, 2000). These findings support inflammation in the respiratory pathways in smokers and notify increased risk of outbreak of such respiratory diseases as COPD and asthma due to tobacco use (Merghani *et al.*, 2012).

Some other studies also report increased production TNF-α and increased serum or plasma concentrations in smokers than non-smokers (Pessina *et al.*, 1993; Tappia *et al.*, 1995; Kuschner *et al.*, 1996) and although similar to the findings of this study, some studies report no significant difference in the inflammatory cytokine between smokers and non-smokers (Keatings *et al.*, 1996; McCrea *et al.*, 1994). Differences in results can be partly attributed to genetic polymorphisms (Merghani *et al.*, 2012). The low levels of these variables being insignificantly different between the two groups could be attributed to the low number of samples in each of the two groups. Otherwise, it is likely that the insignificant difference of this variable is due to the changes in the levels of other inflammatory or anti-inflammatory mediators.

On the other hand, given the findings that TNF-α increases in response to general inflammation in the body and in addition to its secretion by the respiratory tract, some other tissues such as adipose tissue play a major part in its circulating levels, in other words, the bulk of TNF-α is released into the circulation by adipose tissue (Dilyara *et al.*, 2007), it is likely that the inflammation caused by respiratory pathways die to smoking represents only a tiny proportion of the total circulating TNF-α and its higher yet insignificant levels in smokers than non-smokers might be attributed to these factors. Of course, since most scientific studies report the effect of body weight or the percentage of body fat as the most important factors influencing the levels of TNF-α (Mukhopadhyay *et al.*, 2006), it is likely that despite the prolonged smoking cigarette in smoker group, the same percentage of body fat between them and the non-smoker group is involved in the absence of difference in this inflammatory cytokine between the two groups.

If proven it can be concluded from this hypothesis that the effect of smoking on the levels TNF-α in individuals who have the same levels of body fat and visceral obesity is very little, but of course this hypothesis requires further studies with larger sample size along with the control of other intervening variables. Hence measuring the levels of tissual TNF-α in the smooth muscles of the respiratory tract or lung tissue or gene expression or receptor type 1 and 2 of this inflammatory cytokine in smokers could reveal more comprehensive information regarding the impact of smoking on the levels of this cytokine which is of course costly and requires sophisticated experiments.

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