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Comparison of an oxidative stress biomarker "urinary8-hydroxy-2′-deoxyguanosine," between smokers and non-smokers

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Abstract. A great deal of effort has been made on the effect of oxidative stress for smokers. What seems to be lacking, however, is its evidence. Analyzing 1076 participants (age 35.9 ± 12.9, urinary8-OHdG Mean ± S.D., 11.4 ± 6.7, $n = 1076$), our study found the significant increase in a biomarker of DNA damage urinary 8-OHdG/creatinine among smokers (7.75 ± 2.8 ng/ml × CRE ($n = 154$) and 7.36 ± 2.5 ng/ml × CRE ($n = 627$) ($p < 0.05$), Relative Risk = 2.9 (1.4–6.2) sex and age ± 2 matching 105 male smokers and non-smokers. There was no significance on the comparison between female smokers and non-smokers. Smokers have significantly decreased serum alpha-tocopherol (1012 ± 455, 1152 ± 857, $p < 0.03$). The amount of serum ascorbate did not change. Smokers lowered serum HDL-cholesterol compared to non-smokers (59.3 ± 11.8, 63.9 ± 13.3, $p < 0.05$). The result of oxidative stress profile (OSP) also indicated that the increase of oxidative stress to smokers ($p < 0.05$). The calculated value of oxygen radical absorbance capacity (ORAC) of the meal for subjects was 1600 ORAC units.

Keywords: Smokers, urinary8-OHdG (urinary8-hydroxy-2′-deoxyguanosine), oxidative stress profile (OSP), oxygen radical absorbance capacity (ORAC)

1. Introduction

Among 1076 male and female participants, we examined 41 specific biomarkers including urinary 8-OHdG and discussed the relationship between those biomarkers and smoking. A great deal of effort has been made on the effect of oxidative stress for smokers; however, there was no major evidence regarding the relationship of urinary 8-OHdG and smoking.

2. Subjects and method

(1) Analyzing 1076 participants (ranging in age from 18 to 80 years old, Mean ± SD 35.9), we first made 31 items questioning to those participants and then analyzed morning urine, urinary 8-OH d G in ELISA method, serum alpha-tocopherol and ascorbic acid in HPLC method. Other

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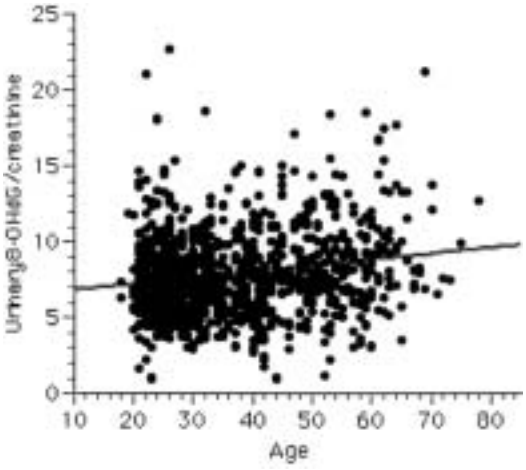

y=0.03589x+6.3741 (P<0.001,n=1076,r^2=0.0359)

+ **ORAC.** (mol Trolox equiv/g)
ORAC (Oxygen Radical Absorbance Capacity) has become a valuable and popular method (B-phycoerythrin (B-PE)) for measuring the antioxidant or radical scavenging capacity of biological and foods samples. Ref. (1)(2) G.Cao, R.L. Prior
++**Input ORAC – output urinary 8-OHdG Model, Ref. (11) (12) (13)**

Fig. 1. Age (18 $\sim$ 80) and urinary 8-OHdG/creatinie.

measurements included serum carotenoid, Vitamin A, folic acid, Vitamin B12, in total 41 items measurements were performed. During the research period, participants took 2300 kcal, and oxygen radical absorbance capacity (ORAC) was 1600 ORAC units per day [1,2].
All biomarker measurement was analyzed by Oxidative Stress Profile (OSP) analysis (JaiCA) [3], which classify those measurements into the oxidative stress index and anti-oxidative index ratio. Also, we performed Chi-square and Mann-Whitney Test among smokers and non-smokers, separately.

(2) 105 male smokers were analyzed by Logistic Regression method after matching in sex and age $\pm$ 2. Significant level was set at 5%. Using SPSS for Mac 10.0 E did all the statistical analysis [4].

3. Results

DNA damage biomarker urinary 8-OHdG (ng/ml) was 11.4 ± 6.7 in average (Mean $\pm$ S.D. $n = 1076$), and urinary 8-OHdG/creatinine level was 7.81 ± 6.67 in 1076 participants. Linear Regression among age from 18 to 80 showed slight increase of urinary 8-OHdG/creatinine level with age ($P < 0.001$, $n = 1076$) (Fig. 1). As shown in Table 1, urinary 8-OHdG/creatinine in smokers and non-smokers were 7.75 ± 2.8 (ng/ml) $N = 154$, and 7.36 ± 2.5 (ng/ml) $N = 627$ ($^*P < 0.05$), respectively. Smokers' urinary 8-OHdG/creatinine level was significantly higher than non-smokers' level. Table 1 also showed urinary 8-OHdG in male group was higher than female group. Urinary 8-OHdG in smoking males was significantly higher than non-smoking males. ($P < 0.05$). Urinary 8-OHdG/creatinine level was also higher than non-smoking male group. ($P < 0.02$). In females, urinary 8-OHdG level was slightly higher in smokers; however, that was not significant.

Table 1
Chi-square and Mann-whitney test was done in smokers and non-smokers

Biomarkers	Smokers (n)	Non-smokers (n)	P value
8-OHdG (ng/ml)	$12.42 \pm 7.0(154)$	$11.0 \pm 6.6(627)$	$P < 0.03$
8-OHdG/creatinine	$7.75 \pm 2.8(154)$	$7.36 \pm 2.5(627)$	$P < 0.05$
Male 8-OHdG	$13.6 \pm 6.5(92)$	$11.5 \pm 6.4(56)$	$P < 0.05$
Male 8-OHdG/creatinine	$7.74 \pm 2.9(92)$	$6.52 \pm 2.1(56)$	$P < 0.01$
Female 8-OHdG	$11.7 \pm 7.3(98)$	$10.9 \pm 6.6(536)$	N.S.
Female 8- OHdG/Weight	$256 \pm 152(98)$	$218 \pm 136(518)$	N.S.

Table 2
OSP of smokers and non-smokers

OSP	Smokers ($n = 137$)	Non-smokers ($n = 608$)	Condition
I	40.2%	28.6%	Dangerous
II	10.2%	21.1%	
III	41.6%	33.7%	
IV	8%	16.6%	Excellent

+OSP is universal oxidative stress analysis. Ref. (3) Y. Yoshikawa.

Table 3
Matching 105 risks of male smokers

Item	Relative risk	95% Cl	P value
OSP I and 8-OHdG	2.9	(1.4–6.2)	$^{*}P < 0.01$

**Risk of smokers was calculated by Logistic Regression Analysis. (SPSS.10 E).

Serum alpha-tocopherol level showed significant decrease in smokers (1012 ± 455, 1152 ± 857, $P < 0.03$). However, serum ascorbic acid level showed no difference between smokers and non-smokers. There were 14.8% of vitamin takers, who stopped taking vitamins one week prior to the test. Serum HDL-cholesterol showed significant decrease in smokers. (59.3 ± 11.8, 63.9 ± 13.3, $P < 0.05$). The Oxidative Stress Profile (OSP) analysis shows there were numbers of smokers in type I 40.1% and non-smokers in type IV 16.6% (see Table 2). This result indicated oxidative damage was significantly higher among smokers than non-smokers. After matching 105 male smokers with non-smokers adjusting in sex and age $\pm$ 2, we calculated the relative risk between smokers and non-smokers. The result was R.R. $= 2.9$ (1.4–6.2, 95% Confidence Interval) ($P < 0.01$) (Table 3).

4. Discussion

In 1076 subjects, it was suggested that urinary 8-OHdG/creatinine damaging level showed increase with aging (Fig. 1). DNA damaging urinary 8-OHdG/creatinine level in male smokers was significantly higher than non-smokers. ($P < 0.05$) It was notified that DNA damaging risk in male smokers was 2.9 (1.4–6.2; 95% CI) times higher. In female group, though 8-OHdG/creatinine level in smokers seemed slightly higher than non-smokers, however it was not significant. There notified a tendency that DNA damaging urinary 8-OHdG/creatinine level in female was lower than male, this is speculated that there is correlation with female hormone [5,6]. Serum alpha-tocopherol level in non-smokers was high, but serum ascorbic acid level was equal between smokers and non-smokers [7,8].

Plasma HDL-cholesterol in smokers was significantly lower than non-smokers, so it was suggested that coronary heart risk is higher than non-smokers [9,10]. According to OSP analysis, it was suggested

that smokers have more oxidative stress than non-smokers. In type I (40.2%), intake of anti-oxidative substance is minor and the environments are in much oxidative damaging load [12,13]. The first definite plan to correct life custom is suggested to stop smoking. ORAC is important in diet, with improvement of anti-oxidative substances such as serum ascorbic acid and serum beta-carotene [11]. Exercises are also recommended to decrease the loaded oxidative stress.

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