Oxidative stress biomarkers and complexity of heart rate variability during acute single cigarette smoking
Original Article

Oxidative stress biomarkers and complexity of heart rate variability during acute single cigarette smoking

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Abstract

Smoking causes sympathovagal change and peripheral vessel injury, which can be assessed by heart rate variability (HRV) that includes endothelial nitric oxide (NO) and sLOX-1 markers, respectively. This study was to investigate HRV and NO change in acute smoking and sLOX-1 overproduction in chronic smoking. A total of 60 males smokers and non-smokers equally were studied. HRV parameters were obtained from all subjects then only smokers were consecutively recorded for 5 minute and 15 minute after completing single smoking and simultaneous blood collection in each segment for NO and sLOX-1 measurement. We found significant lowering of HRV and positive correlation between sLOX-1 and mean arterial pressure in smokers. After 5 minutes of smoking it showed immediate adverse effect with autonomic function represented by HRV and immediate lowering of NO, a vasodilator. Acute smoking influences the autonomic neural control from NO-mediated modulation which can be assessed by noninvasive technique of HRV.

Keywords: heart rate variability, autonomic system, nitric oxide, sLOX-1, smoking
1. Introduction

Cigarette smoking commonly causes heart disease, stroke, chronic obstructive pulmonary disease, cancer, and vascular injury. The duration and number of cigarette smoking are the factors to atherosclerosis. In some occasion sudden cause of death from heart disease is higher in smokers (Erblich, Bovbjerg, & Sloan, 2011; Karakaya et al., 2006; Powell, 1998; Shah & Cole, 2010).

Recently, soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) from serum, which was related with stabilized atherosclerotic plaque formation, was found to increase in cigarette smoker (Takanabe-Mori et al., 2013). Vascular wall inflammation can be induced by oxidative stress from smoking, with a great oxidative combination of low-density lipoprotein (oxLDL) aggregation through major receptor known as lectin-like oxidized low-density lipoprotein (oxLDL) receptor-1 called LOX-1, on the layer of endothelial cells (Moriwaki et al., 1998). LOX-1 is readily released to the blood circulation in sLOX-1 form. The sLOX-1 is excised from ligand specificity of LOX-1. The higher level of sLOX-1 in serum indicates the role of atherosclerotic stenosis, plaque rupture which decreases after gradual cessation of smoking (Takanabe-Mori et al., 2013). Then the plasma sLOX-1 levels were used as predictor of peripheral artery disease (Otsuki et al., 2015), and marker for acute coronary syndrome (Hayashida et al., 2005; Kume et al., 2010).

The epidemiological studies show the correlation of smoking to venous disease of almost forms of arterial disease (Powell, 1998) and inhalation of cigarette smoke has immediate effects on the endothelial blood vessels causing potent vasodilation (Ambrose & Barua, 2004; Barnoya & Glantz, 2005) and also results in systemic release of adrenaline and noradrenaline, to cause muscle contraction and vasoconstriction.
(Powell, 1998). The decreased in endothelial nitric oxide (NO), mediated endothelium-dependent vasodilator, increased the risk of vascular inflammation (Barnoya & Glantz, 2005; Huang & Vita, 2012). Smoking can cause endothelial damage from loss of NO bioactivity in vascular walls that resulted into enhanced platelet aggregation leading to atherosclerotic plaque formation and consequent heart disease and stroke (Ambrose & Barua, 2004; Barnoya & Glantz, 2005; Huang & Vita, 2012).

Sympathetic nerve hyperactivity is also one reason that results in sudden death (Triposkiadis et al., 2009). Enormous increased plasma catecholamines, epinephrine and norepinephrine, from smoking have effects on sympathetic outflow which results into higher blood pressure and heart rate (Karakaya et al., 2006). In addition, there is acute constriction in peripheral blood vessel caused by cigarette smoking (Akishima et al., 2007). Smoking has influenced in brain-heart axis by sympathetic enlargement and decreasing parasympathetic activity measurement through HRV which is an electrocardiographic (ECG) marker reflecting the activity of the sympathetic and vagal components of the autonomic system on the sinus node of the heart. The analysis of HRV performed on ECG recording and comprised the time domain which is assessed by a statistical operation on RR intervals and the frequency domain which is calculated from the power spectral density (PSD) of an array of RR interval (Samuel, Anandhalakshmi, Rekha, & Akhil, 2016). However, the specific autonomic components may not be selective with PSD components, it has been suggested that nonlinear dynamics form HRV should be analyzed (Pitzalis et al., 1996). It is well known that HRV index causes an increase in blood pressure, heart rate, vascular resistance, sympathetic discharge, the decrease in baroreflex activity (Manzano, Vanderlei, Ramos,
& Ramos, 2011) and blunted vagal modulation in heavy smokers (Smith & Fischer, 2001).

Chronic smoking is one of the major coronary risk factor especially in the oxidative damage and has a higher risk than those of non-smoking. Little is known about the association of oxidative stress biomarker with autonomic impairment. In this study, we hypothesized that acute effects of cigarette smoke will increase oxidative stress and it is characterized by decreased endothelial NO with sLOX-1 overproduction and may affect autonomic control. Thus, we investigated the relationships among those parameters in acute smokers and used them for valuable index or indicator of subsequent underlying coronary heart disease which are found normally after atherosclerosis leading to hypertension, arterial stiffness and other vascular diseases.

2. Materials and Methods

A cross-sectional study of 60 non-sick males age over 20 years were participants. Thirty habitual cigarette smoking males who at least 1 cigarette daily and 1 year consecutive smoking and 30 of non-smoking males served as subjects and controls, respectively. All participants were not receiving any medication for any ailment for the last 1 month and gave written informed consent to participate in this study. The research was considered and approved by The Human Ethics Committee, Faculty of Medicine, Thammasat University (MTU-EC-DS-6-068/57).

2.1 Study design

Studies were conducted in a comfortable room and the procedure for performing HRV and blood collection was explained to the subjects in detail. Anthropometric
parameters such as age, height, weight, and smoking status were recorded. All participants rested for 15 minutes in supine position, and ECG were detected by lead II surface with 5 minute recordings. After baseline records were completed, only smokers were asked to smoke a cigarette. Then each 5 minutes segment over 15 minutes of ECG was recorded after complete smoking. Blood serum sample from subjects were collected simultaneously with ECG recording before smoking and then 5 minutes and 15 minutes after complete smoking. After which sLOX-1 and NO were analyzed from smoking serum.

2.2 Analysis of serum sLOX-1 and NO concentration

Blood samples were centrifuged for 15 minutes at 3000 g. Each serum samples were separated and kept in freezer at -20°C until assay. The sLOX-1 serum analysis before smoking was measured using an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of LOX-1 ELISA kit (The RayBio® Human LOX-1/OLR1). This assay employed an antibody specific for human LOX-1 immobilized on well plate. The serum were pipetted into the wells and LOX-1 expressed can be bound by the immobilized antibody then recombinant with anti-human LOX-1 antibodies after that sLOX-1 was assay.

Nitric oxide was measured from the same serum by electrochemistry technique. Serum nitric oxide concentration was detected by using amino-700 nitric oxide sensor (Innovative Instrument, Inc. USA) according to the manufacturer’s instructions. This technique measured nitrite level from electrical current which generate through surface of carbon fiber of electrode. The reaction is followed by oxidizing nitric oxide on microelectrode probe and converted to become nitrite.
2.3 HRV analysis

ECG data were digitized and converted by using LabChart 7, version 7.0.2. The analysis of HRV was performed by Kubios HRV 2.0, (version 2.0). Abnormal beats and artifact areas were excluded. For the time domain, the mean RR interval (RRI), the standard deviation of all RR interval (SDNN), the root mean square of successive RR interval differences (RMSSD), and the proportion of adjacent normal RRIIs differing more than 50 ms in length from the preceding RR (pNN50) were measured. For the frequency domain, the PSD of RR series was obtained through Fast Fourier transformation algorithm. The frequency bands were selected: low-frequency band (LF, 0.04-0.15 Hz), high-frequency band (HF, 0.15-0.4 Hz), and LF to HF ratio (LF/HF). The nonlinear components of HRV were computed. The commonly nonlinear parameters were SD1, SD2, Poincaré plot, α1, α2, and Sample entropy (SampEn).

2.4 Statistical analysis

Results were reported as mean±standard error and percentages. The characteristic of demography and HRV parameters comparing smokers and nonsmokers were obtained by independent-sample \( t \)-test. The NO and HRV parameters alteration after smoking were compared with that of before smoking baseline parameters and analyzed by paired-sample \( t \)-test. The comparison between sLOX-1 and NO are normal and higher mean arterial pressure (MAP) among smokers were obtained by Mann-Whitney U Test. The scatter plots of sLOX-1 and MAP and others correlation between NO and HRV parameters were analyzed by simple linear regression. Only statistically significant of \( p \)-value < 0.05 was considered for all of analysis.
3. Results and Discussion

The general characteristics of subjects were collected by using questionnaires. The summarized information was shown in Table 1. The analyzed factors were age, weight, height, body mass index (BMI), and blood pressure. The average age of thirty male habitual cigarette smokers and thirty age matched of non-smoking was no significant difference including with weight, height, and BMI between the two groups. While both of systolic and diastolic blood pressure (SBP and DBP) was higher in smokers than those of nonsmokers. All of the subjects from this study did not have any major illness in the previous 1 year or used of any drug for the last 1 month.

3.1 Serum oxidative stress markers analysis

The results from Figure 1 showed significant moderate positive correlation between baseline serum sLOX-1 and MAP among smokers in which MAP was calculated as DBP + (SBP-DBP)/3 (Sesso et al., 2000). This result showed that the cigarette smoking may cause increasing oxidative stress and induced inflammatory reaction in blood vessels. In addition, the serum sLOX-1 levels from smokers also correlated with various physiologic markers, such the serum high-sensitivity C-reactive protein (hsCRP) and the expired air carbon monoxide (CO) concentrations (Takanabe-Mori et al., 2013). The baseline serum NO concentrations (55.58±1.42 nmol/L) among the smokers and late-recovery NO after 15 minute smoking (54.47±1.47 nmol/L) were higher than early-recovery NO concentrations after 5 minute smoking (31.21±3.39 nmol/L) (Figure 2). On the serum NO level examination in smokers there was a significant decreased after 5 minute of complete smoking as compared with baseline,
and increased during the 6 to 15 minute period significantly when compared with 5 minute after smoking but insignificant difference with baseline. Figure 2 illustrates the effect of smoking on NO secretion from endothelium, the first inner blood vessel layer controls vascular tone and the vascular inflammatory process (Pittilo, 2000). The decreased in NO after 5 minutes of complete cigarette smoking may show the abnormality in NO mediated endothelium dependent dilation of arterial vessel, leading to vasoconstriction response, increased platelet aggregation, and cell proliferation in the arterial wall. In addition, NO also reacts with superoxide free radical (O$_2^-$) to form peroxynitrite (ONOO$^-$) which is NO-derived RNS (Patel et al., 1999). This dysfunction contributes to atherosclerotic progression (Akishima et al., 2007; Barnoya & Glantz, 2005). The decline of systemic NO may also cause by the decrease of synaptic neurotranmission on the level of medullary neuron that has effect on the electro signal processing of autonomic system (Patel, Li, & Hirooka, 2001).

Among smokers, the results of serum sLOX-1 in case of higher MAP (excess 110 mmHg) group expressed the value higher than those of normal MAP (70 to 110 mmHg) group (Xu et al., 2015). The sLOX-1 results between normal MAP (1.48±0.10 ng/mL) and higher MAP (1.98±0.21 ng/mL) group showed the significant difference with $p$-value < 0.05 whereas, the serum NO from higher MAP group (50.80±1.94 nmol/L) was lower than those of normal MAP group (57.32±1.67 nmol/L, $p$-value < 0.05) (Figure 3). The results in Figure 3 showed that smoking induced inflammatory reaction in blood vessel and increased the risk of cardiovascular incidence. Reactive oxygen species from cigarette combustion is a major antioxidant depletion (Lykkesfeldt et al., 2000) and consequent oxidative stress leading to blood vessel injury by increasing inflammatory marker sLOX-1, this may express the famous indicator of relation
between smoking with inflammation and atherosclerosis (Takanabe-Mori et al., 2013). According to Jaimes (2004), the oxidants in second hand smoke can depress the production of NO. Vasodilator marker by the endothelium independently has an effect on mitochondrial respiration (Jaimes, DeMaster, Tian, & Raij, 2004). In addition, serum sLOX-1 from this study was found to be inversely trending with baseline serum NO concentrations (Figure 3). According to these results smoking is one of the major routes of oxidative stress causing systemic impairment from negative correlation trend between serum sLOX-1 and NO concentrations.

### 3.2 Analysis of heart rate variability

Heart rate (HR) was found to be higher in smokers than in nonsmokers, while RRI, SDNN, RMSSD, pNN50, SD1, and SD2 significantly decreased in smokers. There were no significant difference in both groups of LF, HF, LF/HF, α1, and α2 (Table 2). From this study, we found that power activity of HRV from smokers was significantly lower than nonsmokers because of oxidative stress from long term smoking. The decreasing RRI, SDNN, and other HRV parameters except heart rate among smokers in Table 2 suggests that smoking effect on the sympathovagal modulation resulted to increase sympathetic and decrease parasympathetic activity. It is known that nicotine, free radicals, and CO in cigarette smoke may cause increased activation of the sympathetic nervous system resulting to systemic release of noradrenalin and adrenalin (Papathanasiou1, Mamali, Papafloratos, & Zerva, 2014), thereby increasing heart rate and blood vessel contraction contribute to increase blood pressure.

The HR significantly increased within the first 5 minutes after complete smoking cigarette compared with baseline, and then decreased during the 6 to 15 minute period.
but RRI was contrary with HR exactly. Meanwhile SDNN significantly decreased during the 6 to 15 minute period after smoking. In addition, there was no difference between baseline and after smoking of RMSSD, pNN50 (Table 3). LF and LF/HF significantly increased within the first 5 minutes after complete smoking, and then decreased during the 6 to 15 minute period while HF significantly decreased during 5 minutes after smoking when compared with baseline, and then increased during the 6 to 15 minute period (Table 3). SD2 and α1 significantly increased within the first 5 minutes after smoking a cigarette compared with baseline, and then decreased during the 6 to 15 minute period but SD1 decreased after 5 minute complete smoking and persistent decreased in 15 minutes after smoking. In addition, SD1/SD2 and SampEn significantly decreased within the first 5 minutes after smoking, and then increased during the 6 to 15 minute period (Table 3).

We found an immediate autonomic modulation in cardiac regulation from acute smokers by instantaneous alteration in increased HR and decreased HRV, particularly within the first 5 minutes after a complete cigarette smoking as Karakaya et al found (Karakaya et al., 2007). We observed an decrease in pNN50 after 5 minute of smoking completely and persistent decrease over to 15 minutes, whereas increase in LF and LF/HF in 5 minutes after smoking and return to baseline level after 15 minutes later (Table 3) because LF and LF/HF represent sympathovagal balance of autonomic function meanwhile HF and pNN50 represent parasympathetic nervous system that smoking affected vagal blunt (Cagirci et al., 2009).

An increase in SD2 after 5 minute of smoking completely and recovery of these indices 15 minutes later because SD2 represent sympathetic autonomic function, while SD1 persistently decreased because of smoking effect in sympathovagal balance of
autonomic function by parasympathetic withdrawal and predominant sympathetic activation (Manzano et al., 2011). The α1 was significantly increased immediately after 5 minutes of smoking and showed the change in sympathetic activation or vagal deactivation, in which α1 was due to change in the same direction of peripheral resistance, whereas in α2 there were no significant change because this index was affected by decrease peripheral resistant (Rojo-Alvarez et al., 2007). These α1 and α2 parameters, characterize the intrinsic and extrinsic factors, respectively, in vasomotor tone of cerebral autoregulation (Toth, Rozsa, Springo, Doczi, & Koller, 2011). We believe that there is an intrinsic factor from impairment in endothelial factor such as decreased NO which influences the effect in vasomotor tone regulation. In addition, SampEn relates to a quantity of dynamical systems that can be used to examine physiological systems. Greater regulation systems express in reduction of entropy reflects the sympathovagal balance toward sympathetic predominance (Richman & Moorman, 2000; Rojo-Alvarez et al., 2007). This result shows irregularity in systemic signal alteration from disorder to order immediately after smoking cigarette may have the risk of cardiovascular events (Table 3).

The decreasing ratio of SD1/SD2 during the early recovery period was due to the reduction in SD1 compared with marked increase in SD2 obtained through the Poincaré plot. The characteristics of Poincaré plot is a coordinated plot between current plotted of RRI (RRn) against the previous RRI (RRn+1). Points below the line at 45° to the normal axis called line of identity. A common route to explain the geometry of the Poincaré plot should be fitted to ellipse graph (Karmakar, Khandoker, Gubbi, & Palaniswami, 2009). The ellipse geometry is aligned the position consistent with the identity line as shown in Figure 4. The qualitative analysis of Poincaré plot before and after smoking
during 5 and 15 minutes of completing smoking was shown in Figure 4. During the phase of acute single cigarette smoking, demonstrated by decrease in the dispersion of points at the early recovery phase moments when compared with the basal state and late recovery phase moments. This chart pattern, according to Manzano et al found (Manzano et al., 2011). Our findings show that the qualitative Poincaré plot shape may provide additional insight of smoking on autonomic function.

3.3 Correlation between NO concentration and HRV

The serum NO level in early recovery state (after 5 minute of smoking completely) from smokers showed moderate positive correlation with HF (Figure 5(a)) and significantly irreversible correlation with LF/HF ratio and SD2 from Figure 5(b) and 5(c), respectively. These results showed that endogenously declined NO in blood circulation increases overall sympathetic excitability within the first 5 min after finishing cigarette smoking (Figure 5(b) and 5(c)). The reason for this finding is that smoking affects systemic NOS-inhibition which influence a baroreflex mediated inhibition of sympathetic nerve activity causes increased blood pressure (Middlekauff, Park, & Moheimani, 2014; Narkiewicz et al., 1998). The vascular endothelium and medullary neuron released NO effected in reduction of overall sympathetic or the baroreflex mediated activation of vagal tone elevation (Koch, Hasser, & Schadt, 1995). The overall effect of NO results in a reduction of the efferent outflow (Zanzinger, 1999). In addition, results of circulating NO is also shown with significantly correlation with SampEn in Figure 5(d). The algorithm of SampEn is the signal complexity measuring the time sequence, especially it can be applied to clinical data of noisy time series (Yuanyuan, Chengyu, Binhua, & Mengsun, 2014). Smoking might induce
sympathetic vasomotor activity and heart rate resulting to increase sympathetic dominance on the cardiac autonomic nerve activity. The change of SampEn was displayed in these modulations. The greater complexity of SampEn indicates physiological mechanism of systemic disorder that ranges from order to disorder manner (Richman & Moorman, 2000). Thus the result of SampEn from this study unsurprisingly showed significantly positive correlation with NO.

4. Conclusions

The effects of cigarette smoking was examined from this study through the elevated vascular injury marker, sLOX-1 and autonomic modulation presented by HRV. In conclusion, lower NO, which represents vascular oxidative stress marker causes an acute effect on single smoking, as obviously seen. Moreover, the overproduction of sLOX-1 and decreasing of NO bioavailability may affect the autonomic control and induced vascular lesion. Most importantly, the results of the data analysis from HRV measurements indicates that autonomic impairment occurring in chronic smokers and top up during 5 minutes after completing a single cigarette smoking, which elevates sympathetic activity and blunts vagal tone immediately. Furthermore, distribution of Poincaré plot also provides a sensible and simple tool to detect immediate change in HRV from acute smoking.

Acknowledgments

The author gratefully acknowledge the financial support provided by Thammasat University Research Fund under the TU Research Scholar, Contract No. 56/2557 and very special thanks for the supporting from the Tobacco Control Research
and Knowledge Management Center (TRC) and Thai Health Promotion Foundation, which enabled me to undertake this study.

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Table 1. The general characteristic of participants.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-smokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.8±9.11</td>
<td>35.4±10.21</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.57±15.35</td>
<td>64.27±10.40</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.67±5.83</td>
<td>167.93±5.73</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.13±4.44</td>
<td>22.78±3.46</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>123.03±11.17</td>
<td>133.80±15.04†</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.27±11.47</td>
<td>82.33±9.73*</td>
</tr>
</tbody>
</table>

Significance compared with nonsmoker; *p-value < 0.05 and †p-value < 0.01
Table 2. Heart rate variability (HRV) data of participants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonsmokers</th>
<th>Smokers</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>70.93±1.82</td>
<td>85.54±2.74</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RRI (ms)</td>
<td>864.07±21.69</td>
<td>725.81±25.36</td>
<td>0.0001</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>33.85±2.64</td>
<td>22.57±2.45</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>35.57±3.31</td>
<td>22.56±3.06</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>15.88±3.11</td>
<td>6.34±1.80</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>LF (n.u.)</td>
<td>54.00±92.92</td>
<td>57.14±3.04</td>
<td>NS</td>
</tr>
<tr>
<td>HF (n.u.)</td>
<td>45.99±89.45</td>
<td>42.86±3.04</td>
<td>NS</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>1.41±0.16</td>
<td>1.99±0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Total power (ms²)</td>
<td>1227.13±181.41</td>
<td>614.23±120.71</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SD1 (ms)</td>
<td>25.63±2.44</td>
<td>16.08±2.18</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td>61.07±4.83</td>
<td>40.71±3.49</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>α1</td>
<td>1.04±0.04</td>
<td>1.12±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>α2</td>
<td>0.90±0.03</td>
<td>0.91±0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant
Table 3. The alteration in the time domain, frequency domain and nonlinear parameters of heart rate variability after smoking a cigarette.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basal state</th>
<th>Early recovery</th>
<th>Late recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(5 min after smoking)</td>
<td>(15 min after smoking)</td>
<td></td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>85.54±2.73</td>
<td>92.47±2.55*</td>
<td>90.46±2.54**</td>
</tr>
<tr>
<td>RRI (ms)</td>
<td>725.81±25.36</td>
<td>666.43±19.79*</td>
<td>680.65±20.03**</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>22.57±2.45</td>
<td>22.34±2.47</td>
<td>20.09±2.29**</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>22.65±3.06</td>
<td>18.37±3.12</td>
<td>18.87±3.32</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>6.34±1.80</td>
<td>4.84±2.06</td>
<td>4.31±2.20</td>
</tr>
<tr>
<td>LF (n.u.)</td>
<td>57.14±3.04</td>
<td>71.81±2.27*</td>
<td>62.85±3.13**</td>
</tr>
<tr>
<td>HF (n.u.)</td>
<td>42.86±3.04</td>
<td>28.19±2.27*</td>
<td>37.15±3.13**</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.99±0.39</td>
<td>3.58±0.53*</td>
<td>2.73±0.50**</td>
</tr>
<tr>
<td>SD1 (ms)</td>
<td>16.08±2.18</td>
<td>14.26±2.29</td>
<td>13.46±2.36</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td>40.71±3.49</td>
<td>49.84±4.38*</td>
<td>39.24±3.68†</td>
</tr>
<tr>
<td>SD1/SD2</td>
<td>0.37±0.03</td>
<td>0.27±0.02*</td>
<td>0.32±0.02†</td>
</tr>
<tr>
<td>α1</td>
<td>1.12±0.05</td>
<td>1.34±0.04*</td>
<td>1.20±0.05†</td>
</tr>
<tr>
<td>α2</td>
<td>0.91±0.04</td>
<td>0.90±0.04</td>
<td>0.94±0.03</td>
</tr>
<tr>
<td>SampEn</td>
<td>1.47±0.07</td>
<td>1.21±0.07*</td>
<td>1.38±0.06†</td>
</tr>
</tbody>
</table>

*; significance compared with basal state, †; significance compared with early recovery.

**; significance compared with both basal state and early recovery.
Figure 1. Serum sLOX-1 levels showed moderate positive correlation with mean arterial pressure.

\[ r = 0.60, \ p\text{-value} = 0.001 \]
Figure 2. The change in serum nitric oxide (NO) of smokers after smoking a cigarette,

* $p$-value $< 0.001$. 
Figure 3. The serum sLOX-1 and NO comparison in normal and higher MAP group from smokers, * $p$-value < 0.05

<table>
<thead>
<tr>
<th>Basal state</th>
<th>After smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early recovery (5 min)</td>
<td>Late recovery (15 min)</td>
</tr>
</tbody>
</table>

Figure 4. Distribution of Poincaré plots of smoker through the phase of smoking a cigarette.
Figure 5. Correlation between circulating NO concentration of smokers and HF (a), LF/HF ratio (b), SD2 (c), and SampEn (d) in early recovery state.