

*Full Length Research Paper*

# Correlation between pulse wave velocity and serum sLOX-1 in patients with acute cerebral infarction

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Accepted 24 August, 2011

To investigate the relationship between pulse wave velocity (PWV) and serum lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) level in patients with acute cerebral infarction, a total of 58 patients with acute cerebral infarction occurring within 72 h and 30 subjects (control) without stroke receiving physical examination were recruited into the present study. The right carotid-femoral PWV (cfPWV) was measured and serum sLOX-1 level was determined by ELISA. The PWV and serum sLOX-1 level in patients with acute cerebral infarction were significantly higher than those in the control group (PWV:  $11.69 \pm 2.56$  m/s Vs.  $9.60 \pm 1.92$  m/s,  $P < 0.05$ ; sLOX-1:  $42.92 \pm 6.88$  mg/L Vs.  $36.03 \pm 4.70$  mg/L,  $P < 0.01$ ). No marked difference was observed in the PWV and serum sLOX-1 level among patients with different infarction sizes. Correlation analysis revealed PWV was positively correlated with the serum sLOX-1 level ( $r = 0.579$ ,  $P < 0.01$ ). PWV has favorable consistence with sLOX-1 used as a non-invasive method to evaluate the degree of atherosclerosis in patients with acute cerebral infarction. PWV and sLOX-1 may have important clinical implications in the prevention and treatment of cerebral infarction.

**Key words:** Cerebral infarction, atherosclerosis, pulse wave velocity, lectin-like oxidized low-density lipoprotein receptor-1.

## INTRODUCTION

Acute cerebral ischemia is one of the most frequently encountered diseases in clinical practice with great mobility and mortality. As one of the most important risk factors of ischemic stroke, atherosclerosis (AS) may result in stenosis and hardening of the artery wall. Among the parameters reflecting the compliance of artery wall, pulse wave velocity (PWV) may serve as a non-invasive measure and can be used for bedside examination. PWV is the pulse speed generated by the heart beat diffusing on the artery wall. It is determined by the mechanical characteristics (stickiness and elasticity) and geometrical characteristics (diameter and artery wall thickness) of artery wall and the density of blood. Studies indicated that PWV can reflect the compliance of artery wall when the effects of confounding are controlled: the higher the PWV, the worse the compliance of artery wall (Avolio et al., 2002). Lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) is one of the receptors of oxidized-low

density lipoprotein (ox-LDL) (Sawamura et al., 1997) and plays an important role in the development of AS.

Soluble LOX-1 (sLOX-1) is the extracellular part of LOX-1 which is released into the blood due to the cleavage by protease. sLOX-1 level can reflect the expression of LOX-1 (Kume and Kita, 2001). LOX-1 involves the occurrence and development of AS, and PWV can reflect the early changes in the compliance of blood vessel wall due to AS. Thus, there might be a correlation between sLOX-1 level and PWV during the acute cerebral infarction. The aim of this study was to demonstrate PWV as a non-invasive method to reflect the AS through analyzing the correlation between PWV and sLOX-1 and to investigate the relationship between PWV and serum sLOX-1 level in acute cerebral infarction patients.

## MATERIALS AND METHODS

### Subjects

A total of 58 patients with acute cerebral infarction occurring within

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**Table 1.** General information of subjects from two groups.

Variable	Cerebral infarction group	Control group
Number	58	30
Age (y; $\bar{x}\pm s$ )	63.0 $\pm$ 10.4	61.2 $\pm$ 10.7
Male (%)	42(72.41)	22(73.33)
Risk factors (%)		
Hypertension	39(67.24)*	11(36.67)
Diabetes	16(27.59)	4(13.33)
Smoking	30(51.72)	14(46.67)
Alcohol drinking	24(41.38)	10(33.33)
Obesity	42(72.41)*	12(40.00)
Hypercholesterolemia	13(22.41)	2(6.67)
Lipide classify ( $\bar{x}\pm s$ )		
LDL-C, mmol/L	3.05 $\pm$ 0.94*	2.20 $\pm$ 0.86
HDL-C, mmol/L	1.11 $\pm$ 0.28	1.43 $\pm$ 0.26
TG, mmol/L	1.43 $\pm$ 0.91	1.25 $\pm$ 0.68
LP(a), mg/L	331.93 $\pm$ 262.25*	123 $\pm$ 78.35

\*P<0.01 Vs. control group.

72 h and 30 subjects without stroke receiving physical examination were recruited from the Department of Neurology of the Second Hospital of Shandong University from November 2007 to March 2008. The diagnosis of cerebral infarction was based on the criteria for cerebrovascular disease developed in the 4<sup>th</sup> National Cerebrovascular Disease Conference (Millasseau et al., 2005) and confirmed by brain CT or MRI. There were 42 males and 16 females in cerebral infarction patients with a mean age of 63.0  $\pm$  10.4 years (range: 38 to 80 years). According to the infarction size which was measured by brain CT or MRI that showed the infarction of the largest cross-section, these patients were subdivided to three groups: lacunar infarction group (n = 30; <1.5 cm in diameter), general infarction group (n = 21; 1.5 to 4 cm) and severe infarction group (n = 7; > 4 cm). Subjects without stroke served as controls group, among subjects with a history of stroke or having positive neurological signs are excluded. There were 22 males and 8 females in the controls with a mean age of 61.2  $\pm$  10.7 years (42 to 80 years). There is no significant difference in the gender and age between two groups. Informed consent was obtained before study and these subjects received free examination. General information such as age, sex, weight, blood pressure, smoking, alcohol drinking, etc was recorded. There is a description of diabetes on a chart, but there is no separate article on the study of atherosclerosis caused by diabetes. The study has been approved by the Ethics Committee of Second Hospital of Shandong University.

#### Measurement of PWV

Before PWV measurement, blood pressure was routinely measured in the morning in the fasting state. The right cervical-femoral PWV (cfPWV) was measured using Complior SP (Artech-Medical, France). The PWV was calculated from the measurements of the pulse transit time (T) and the distance travelled by the pulse between the two recording sites as follow: PWV=L (m)/ T(s). Subjects lied in a supine position and the transducers were independently placed on the carotid artery and femoral artery with

obvious pulse the distance between which was measured and input into a computer. Measurement was carried out ten times and the data were averaged as the cfPWV.

#### Detection of serum SLOX-1

The fast venous blood was collected and the sLOX-1 level measured by ELISA (rat anti-human soluble LOX-1 ELISA, USA).

#### Statistical analysis

Statistical analysis was performed using SPSS version 13.0 and  $\chi^2$ -test, t-test, analysis of variance and linear correlation analysis were applied for comparisons. The quantitative data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). A value of P < 0.05 was considered statistically significant.

## RESULTS

### General information

The general information is shown in Tables 1 and 2. The subjects in the control group had no history of cerebrovascular disease but associated with other diseases which had been listed in Table 1. No significant differences were found in the gender and age between cerebral infarction group and control group. However, statistically significant differences were found in the risk factors such as hypertension, obesity, LDL-C, LP (a) between two groups. In addition, there were no marked differences in the important parameters among three

**Table 2.** General information of cerebral infarction patients.

Variable	Lacune infarction	General infarction	Severe infarction
Sample	30	21	7
Age (y; $\bar{x}\pm s$ )	63.2 $\pm$ 9.8	61.5 $\pm$ 13.4	66.4 $\pm$ 8.4
Male (%)	25(83.33)	14(66.67)	5(71.43)
Risk factors (%)			
Hypertension	24(80.00)	19(90.48)	4(57.14)
Diabetes	8(26.67)	4(19.05)	5(71.43)
Smoking	19(63.33)	11(52.39)	4(57.14)
Alcohol drinking	15(50.00)	11(52.39)	3(42.86)
Obesity	19(63.33)	19(90.48)	4(57.14)
Hypercholesterolemia	7(23.33)	4(19.05)	2(28.57)
Lipide classify ( $\bar{x}\pm s$ )			
LDL-C (mmol/L)	3.08 $\pm$ 0.97	3.29 $\pm$ 0.93	3.06 $\pm$ 1.06
HDL-C (mmol/L)	1.14 $\pm$ 0.29	1.21 $\pm$ 0.39	1.12 $\pm$ 0.48
TG (mmol/L)	1.48 $\pm$ 0.90	1.31 $\pm$ 0.99	2.00 $\pm$ 2.45
LP(a) (mg/L)	317.79 $\pm$ 261.16	256.06 $\pm$ 175.59	467.67 $\pm$ 318.69

No statistically significant differences were observed among subgroups in the variables above.

**Table 3.** PWV and serum sLOX-1 level in two groups.

Variable	Cerebral infarction group	Control group
sLOX-1 (mg/L)	42.92 $\pm$ 6.41**	36.03 $\pm$ 4.70
PWV (m/s)	11.69 $\pm$ 2.56*	9.60 $\pm$ 1.92

\*\*P<0.01 vs. control group; \*P<0.05 vs. control group.

**Table 4.** PWV and serum sLOX-1 level in subgroups.

Variable	Lacunar infarction	General infarction	Severe infarction
sLOX-1 (mg/L)	42.69 $\pm$ 6.88	43.77 $\pm$ 4.88	41.46 $\pm$ 8.44
PWV (m/s)	11.42 $\pm$ 2.82	12.02 $\pm$ 2.36	11.86 $\pm$ 2.11

No statistically significant differences were observed in the PWV and serum sLOX-1 among subgroups.

subgroups in cerebral infarction group.

### PWV in different groups and subgroups

As shown in Table 3, the PWV was 11.69  $\pm$  2.56 m/s in cerebral infarction patients and 9.60  $\pm$  1.92 m/s in controls showing significant difference (P < 0.05). As shown in Table 4, the PWV was 11.42  $\pm$  2.82 m/s in the lacunar infarction group, 12.02  $\pm$  2.36 m/s in the general infarction group and 11.86  $\pm$  2.11 m/s in the severe

infarction group revealing no marked difference (P > 0.05).

### SLOX-1 in different groups and subgroups

As shown in Table 3, the SLOX-1 level was 42.92  $\pm$  6.88 mg/L in cerebral infarction group and 36.03  $\pm$  4.70 mg/L in the control group showing dramatic difference between groups (P < 0.01). As shown in Table 4, the serum sLOX-1 level was 42.69  $\pm$  6.88 mg/L in the lacunar

infarction group,  $43.77 \pm 4.88$  mg/L in the general infarction group and  $41.46 \pm 8.44$  mg/L in severe infarction group revealing no marked difference ( $P > 0.05$ ).

### Correlation between PWV and serum sLOX-1

Correlation analysis showed the correlation coefficient between PWV and serum sLOX-1 was 0.579 in acute cerebral infarction patients ( $P < 0.01$ ). In addition, we did not analyze the correlation between PWV and serum sLOX-1 in other various risk factors separately in the control subjects.

## DISCUSSION

According to the Moens-Korteweg Equation (Millasseau et al., 2005), PWV is positively proportional to the square root elasticity coefficient (Millasseau et al., 2005). In the AS, the arterial elasticity is compromised and the conduction velocity of pulse wave in the artery wall correspondingly increases. Several studies have indicated that the PWV can be used as a marker of arterial stiffness with high sensitivity and repeatability. Thus, it is applicable in clinical trials with large population and those with interventions. The arterial stiffness is correlated with age, sex, smoking, hypertension, diabetes, hypercholesterolemia, etc. Rotterdam et al showed that the arterial stiffness was closely related to the AS (van Popele et al., 2001). Epidemiological studies and clinical trials also reveal that AS is an independent risk factor of cardiovascular diseases (Blacher et al., 1999). Nakano et al. (2004) reported in a longitudinal study that the arterial stiffness which was evaluated by PWV was an independent risk factor of fatal stroke in patients with primary hypertension.

LOX-1 is a type II single-stranded transmembrane protein and there are two splice sites in the extracellular part of this protein: arg86-Ser87 and Lys89-Ser90. In the presence of serine protease, LOX-1 is cut and released into the blood forming sLOX-1. Generally, the acceptor of sLOX-1 competitively inhibits the sLOX-1. However, no studies have revealed sLOX-1 can competitively inhibit the binding of ox-LDL to LOX-1. This may be attributed to the low affinity between ox-LDL to sLOX-1 and/or multiple binding sites in the ox-LDL (Murase et al., 2000). The serum sLOX-1 level is positively correlated with the LOX-1 on the cell membrane. Thus, the sLOX-1 may become a novel marker for the diagnosis of AS and LOX-1 related diseases (Kume and Kita, 2001; Navarra et al., 2010).

LOX-1 plays an important role in the occurrence and development of AS (Kita et al., 2001). Firstly, ox-LDL involves in the occurrence of AS (Kita et al., 2001). As a main receptor of ox-LDL, LOX-1 mediates the majority of

the toxic effects of ox-LDL. Secondly, the LOX-1 is highly expressed in the artery with AS and mainly expressed in the three types of cells: macrophages, SMC and vascular endothelial cells (Sawamura et al., 1997; Eto et al., 2006) which are closely related to the AS. Thirdly, the up-regulated LOX-1 mediates a series of pathophysiological processes in AS: it can mediate the interaction between platelets and endothelium as a cellular adhesion molecule and it is relevant with the endotoxin related inflammatory response which may initiate and promote the AS (Kakutani et al., 2000). Fourthly, the anti-AS drugs including statins can inhibit the atherosclerogenic factors induced up-regulation of LOX-1, which may be one of the beneficial effects of statins (Mehta et al., 2001). Recently, a study on a rabbit model of hypercholesterolaemia shows that LOX-1 is highly expressed in the unstable atherosclerotic plaques, which confirms the relationship between LOX-1 and instable atherosclerotic plaques (Ishino et al., 2007).

AS is a pathological manifestation which is not confined to a certain site but involves arteries in a variety of tissues/organs. AS can directly result in change in vascular elasticity and increase the vascular stiffness (Graham et al., 2008). In the present study, PWV was non-invasively measured. Our results showed PWV could favorably reflect the arterial stiffness and then reflect the degree of AS. Our findings revealed the PWV was significantly increased in acute cerebral infarction patients when compared with controls. This result indicates cerebral infarction patients not only have severe AS in the intracranial blood vessels but have significantly increased vascular stiffness and systemic AS when compared with those without stroke. Of note, the occurrence and development of AS in the intracranial blood vessels and blood vessels are synchronous.

In the present study, the serum sLOX-1 level in the cerebral infarction group was dramatically higher than that in the control group, indicating LOX-1 is related to the AS induced cerebral infarction. LOX-1 is highly expressed in the atherosclerotic blood vessels and largely expressed in the endothelial cells, smooth muscle cells, macrophages, etc. LOX-1 is not a molecule with constitutive expression and transiently expressed in the presence of inducing factor including ox-LDL, inflammatory factors, shearing force, etc (Murase et al., 1998). The main pathological process involving in the AS induced cerebral infarction is thrombosis and a key structure is intravascular unstable plaques. It has been confirmed that LOX-1 is relevant with the instability of plaques (Kakutani et al., 2000). Thus, during the cerebral infarction, LOX-1 is highly expressed in the presence of inducing factors, which is characterized by the increase of serum sLOX-1. In addition, our results revealed no significant difference in serum sLOX-1 level among patients with different infarction sizes. This may be attributed to that embolus with same size may result in different infarction size in different cerebral blood vessels.

However, serum LOX-1 may be related to the initial size of plaques, but not with the secondary infarction size.

Our results reveal that PWV is correlated significantly with serum sLOX-1 level in the cerebral infarction patients, which suggests PWV and sLOX-1 play similar important role in the evaluation of AS degree and acute cerebral infarction

Taken together, acute cerebral infarction patients have both elevation of PWV and increase of serum sLOX-1 level, and correlation between PWV and serum sLOX-1 level is observed among these patients. These findings suggest PWV and sLOX-1 can be used to reflect the degree of AS in acute cerebral infarction patients with favorable consistence. Detection of PWV and sLOX-1 may have clinical implications in the prevention and treatment of cerebral infarction but more studies are required to confirm our results. Our findings may provide novel tool for the evaluation of AS degree in cerebral infarction patients.

## ACKNOWLEDGEMENTS

This study was supported by the fund from Shandong Province Department of Education. No potential conflicts of interest relevant to this article were reported.

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