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Original Article

Effects of percutaneous lower-extremity arterial interventions on endothelial function and inflammation response in patients with both type 2 diabetes and lower-extremity peripheral arterial disease

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Abstract: Background: The high incidence and damage of PAD in people with diabetes has aroused wide attention. We aimed to examine effects of percutaneous lower-extremity arterial interventions (PLEAIs) on endothelial function and inflammation response in type 2 diabetes (T2D) patients with lower-extremity peripheral arterial disease (PAD). Methods: 78 T2D inpatients with PAD were selected into the treatment group. Their venous levels of von Willebrand Factor (vWF) and high sensitivity C reactive protein (hsCRP) were measured. Blood samples were collected from the arterial sheath for vWF and hsCRP tests. Venous levels of vWF and hsCRP were monitored at 24 hours, 48 hours, 1 week, and 2 weeks post PLEAIs. Results: Prior to PLEAIs, venous levels of vWF and hsCRP in the treatment group were significantly higher than the control group. The arterial levels of vWF and hsCRP were 117.9%±15.1% and 5.19±0.76 mg/L in the control group, while those levels in the treatment group before intervention were also significantly higher than in the control group. In the treatment group prior to inventions, vWF and hsCRP levels of arterial ischemic regions were significantly higher than the non-ischemic regions. The vWF level of arterial ischemic regions after treatment was significantly higher than that prior to treatment. Conclusions: PLEAIs applied to those patients may lead to worse endothelial dysfunction and activated inflammatory response during treatment and 1 week after treatment, which indicates an emerging necessary of early protection or care on endothelial function and inflammatory reaction during and post PLEAIs.

Keywords: Type 2 diabetes, lower-extremity peripheral arterial lesions, percutaneous lower-extremity arterial intervention, von Willebrand Factor, high-sensitivity C reaction protein

Introduction

Lower-extremity peripheral arterial disease (PAD) is an abnormal condition characterized by vascular occlusion or narrowing in lower limb arteries. There is increasing evidence suggesting that people with diabetes, particularly type 2 diabetes (T2D), are much more likely to develop PAD than the general population [1-4]. At present, PAD is thought to be a major cause of diabetic foot ulcer and lower extremity amputation in patients with both T2D and PAD [5-7]. The high incidence and damage of PAD in people with diabetes has stirred up wide attention on the study of PAD in the diabetic condition. The specific clinical guidelines regarding the care of patients with both diabetes and PAD are kept updating with the base of relevant evolving studies [4, 8, 9], which are also accompanied with efficient and advanced treatment techniques for those patients, such as percutaneous lower-extremity arterial interventions (PLEIs). PLEI technologies, including percutaneous transluminal angioplasty and stent replacement, have largely improved the condition of PAD in diabetes patients, especially when lifestyle modulations and medication therapy fail [7, 10, 11].

Accumulating evidence implies that the PAD biology in diabetes is unique and much unknown [10, 12]. The involvement of endothelial function and inflammatory response plays an important role in the development of PAD in patients

Table 1. Baseline characteristics of subjects with T2D

Variables	Treatment group n=78 (median)	Control group n=42 (median)	*P*
Male (%)	60 (76.9)	29 (69.0)	0.347
Age (year)			0.792
Median	70.62±7.63	68.87±8.30	
Extremum	54, 85	58, 82	
T2D age (year)			0.951
Median	16.67±5.61	17.42±5.28	
Extremum	7, 30	8, 27	
Complications (%)			
Hypertension	45 (57.7)	26 (61.9)	0.654
Coronary disease	33 (42.3)	14 (33.3)	0.337
Cerebral infarction	23 (29.5)	9 (21.4)	0.341
Dyslipidemia	52 (66.7)	27 (64.3)	0.793
Retinal vascular lesions	11 (14.1)	7 (16.7)	0.708

with diabetes [13, 14]. However, global changes of these involved factors post PLEIs or acute regional effects of PLEIs on endothelial function and inflammatory response of arterial ischemic sites remain poorly characterized. von Willebrand factor (vWF) has been widely used to assess the endothelial function in patients with diabetes [13, 15-17], while high-sensitivity C reaction protein (hsCRP) has been regarded as a standard marker of inflammation reaction for several decades [18, 19]. This study is aimed to characterize the effects of PLEAIs in patients with both T2D and PAD by monitoring plasma levels of vWF and hsCRP prior to or post treatment, which may provide critical evidence for a better care of T2D patients with PAD or according update of future intervention guidelines.

Methods

Study subjects

From May 2008 to December 2012, 78 inpatients with diabetic PAD who gained success of PLEIs were recruited into our treatment group, including 60 males and 18 females with age range from 54 to 85 years old (median: 72). T2D of the subjects was diagnosed according to the 1997 American Diabetes Association Criteria [20], whose diabetes ages were 7 to 30 years (median: 16). Lower-extremity PAD of subjects was classified following the Fontaine classification standard, 29 patients at stage II b and 49 at stage III included. Among them, 45 patients have hypertension, 33 with coronary artery disease, 52 with dyslipidemia, 3 post iliofemoral bypass graft, 2 with vascular narrowing after femoral arterial stent implantation, and 11 with retinal vascular lesions. At the same hospitalization period with the treatment group, 42 T2D patients with normal lower extremity arteriography were chosen as control group of this study. The control group consisted of 29 males and 13 females, aged from 58 to 82 years (median: 71) and with T2D history of 8-27 years (median: 15). Among the control subjects, 26 of them have hypertension, 14 with coronary artery disease, 9 with cerebral infarction, 27 with dyslipidemia, and 7 with retinal vascular lesions. There is no statistically significant difference on the baseline characteristics of the treatment and control groups, based on all the information we have collected so far ($P>0.05$) (**Table 1**).

Ethics statement

This study protocol gained official approval from Ethics Committee of the Fourth Hospital of Hebei Medical University. Written informed consent was obtained from all participants in advance of all sample collections.

Clinical examinations

The clinical observations were performed according to the following indexes:

1) Success standard of PLEIs: at least 1 site of blood vessel occlusion was eliminated or the vascular narrowing was widened to less than 30%, either of which has ensured the blood flow to the bottom of foot. 2) Clinical efficacy: limb pain, cold sensitivity, walking distance, ulcer healing improvement and ankle-brachial pressure index (ABI) were observed or measured in the subjects prior to or 1 week after PLEIs. 3) Complications: the attentions post treatment was made on relevant complications, including hematoma around the puncture position, angiorrhexis, pseudoaneurysm, formation of arterial dissection or cardiovascular and cerebrovascular accident. 4) Follow-up

Table 2. Fontaine classifications of participants prior to and post treatment

	Fontaine classification				*P*
	I or less	II a	II b	III	
Before treatment	0	0	29	49	<0.01
After treatment	11	23	37	7	

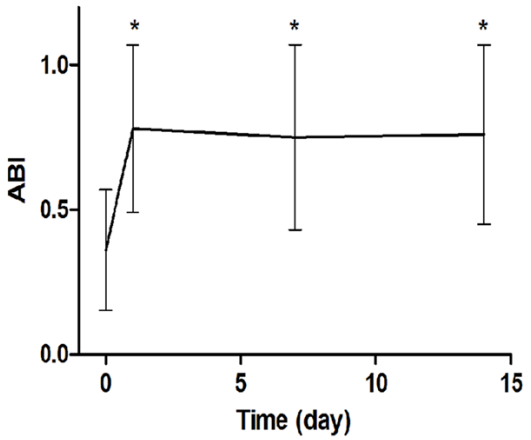

Figure 1. Changes of ABI post treatment in the treatment group. The star sign * indicates *P*<0.01 compared to pre-treatment level.

observation: A clinical examination schedule was made to investigate the short-term and long-term effects of PLEIs in all treatment subjects, which are still being carried on as of the time writing up this manuscript. They are being examined in 1 month, 3 months, 6 months and then each year till 5 years after operations.

Routine examinations prior to PLEIs were applied to all subjects, included with tests on cardio-pulmonary function, hepatonephric action, urine, blood, ABI, and lower-extremity computed tomography angiography. Prior to and 1 week after PLEIs, medications routinely taken were kept in use by subjects, such as insulin for glycemic control, simvastatin (Zocor, Hangzhou MSD Pharmaceutical Co Ltd) for lipid modulation, and benazepril hydrochloride (Lotensin, Beijing Novartis Pharmaceutical Co Ltd) for hypertension treatment. Medications to benefit further treatment were taken by treatment subjects 1 week before PLEIs, including Aspirin enteric-coated tablets (Bayer Pharmaceuticals Ltd of Germany) with 100 mg/day for long-term use and Clopidogrel (Boliwei, Hangzhou Sanofi Pharmaceuticals Ltd) with 75 mg/day continued using for 6 months. At 6:00 AM of the day when PLEIs were carried out, the fasting blood from elbow vein was drawn as venous blood samples before treatment for vWF and hsCRP measurement. The artery blood samples before treatment was collected right after the success of placing catheter sheath into femoral artery of treatment subjects under regional anesthesia. Blood collected from distal ischemic sites of the artery with occlusion or narrowing was used as sample of arterial ischemic region before the intervention. At the same location, blood specimens were taken directly after successful balloon dilatation or stent implantation as samples of arterial ischemic site after intervention. For the prevention of blood clotting, 5 ml of heparin saline (heparin, 25 U/ml) was injected into the arterial sheaths instantly after drawing the samples. In the meanwhile, venous injection of heparin (3000 U) was given with an addition of 1000 U every hour until the operation was done. Hypodermic injection of low molecular heparin was also performed to prevent clotting in the treatment subjects every 12 hours until 14 days after operation, 5000 U/time. Post treatment samples were collected from elbow veins of subjects at 24 hours, 48 hours, 7 days and 14 days after surgery. Blood specimens from the vein and artery of control subjects were also collected as pre-treatment control. All blood samples were collected with 3 ml from indicated locations at the suggested time and immediately were spun down at 1500 rpm for 5-10 min. The supernatants of all samples were collected and kept in 4°C for vWF and hsCRP tests. vWF levels were measured with a commercial kit from Shanghai Huole Bio-tech Inc based on the principle of double antibody sandwich enzyme-linked immunosorbent. hsCRP concentrations were determined by high sensitivity photoextinction analysis, reagents and equipments for which all are purchased from American Beckman Coulte Inc. All the procedures were performed strictly according to manuals of the utilized kits.

Statistical analyses

SPSS13.0 software package was used for statistical analysis of this study. All the measurement data was presented as mean ± standard deviation ($\bar{x} \pm s$). *t* test was applied to compare the means of two groups. Changes before and after treatment was analyzed with paired t test, for comparison of which x^2 test was also applied. P<0.05 indicates the existence of significant difference for all data analysis.

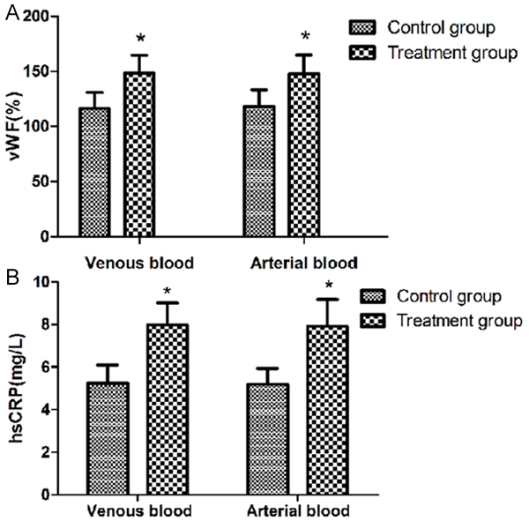

Figure 2. A. Comparison of plasma vWF levels between pre- and post- treatment. *P<0.01. B. Comparison of plasma hsCRP levels between pre- and post intervention. *P<0.01.

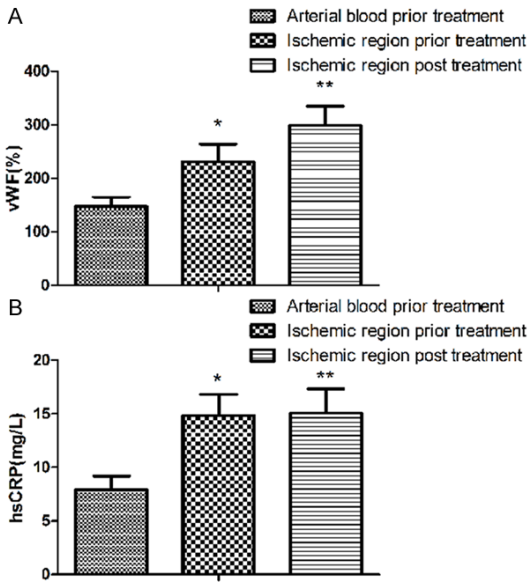

Figure 3. A. Plasma vWF level changes by PLEI in the artery and ischemic sites of treated subjects. *P<0.001 (arterial changes), **P<0.01 (ischemic regional changes). B. Plasma hsCRP level changes by PLEI in the artery and ischemic sites of treated subjects. *P<0.01 (arterial changes), **P=0.451 (ischemic regional changes).

Results

Certain levels of intermittent claudication and rest pain were improved by PLEIs in our treatment subjects with gradual recovery of ulcer and infection. Stages of blood vessel occlusion or narrowing in the treatment subjects were assessed and re-classified by Fontaine classification's standard 1 week after treatment, the overall comparison of which with pre-treatment has been significantly improved (χ^2=66.46, P<0.01). The number of treated patients at Fontaine III was significantly decreased from 49 to 7 (**Table 2**). The positive effect of PLEIs on treatment participants was also indicated by the significant increase of mean ABI from 0.36±0.21 of pre-treatment to 0.78±0.29 of 24 hours post treatment, 0.75±0.32 of 7 days post treatment and 0.76±0.31 of 14 days post treatment (comparison of the post and pre-treatment, all P<0.01, **Figure 1**). During years' follow-up visits after PLEIs, 1 subject died of acute myocardial infarction in 3 months, 1 death caused by cardiac failure in 1 year. Three subjects gained success in the second operation, including 1 with in-stent restenosis 3 years and 2 months after femoral artery stent implantation and the other 2 arterial restenosis in 2.1 years and 2.3 years respectively after balloon dilation treatment in Inferior genicular artery. In the whole follow-up visit till now, no abnormal changes occurred in participants of the control group.

In control subjects, both vWF and hsCRP levels showed no significant difference between vein and artery blood samples (**Figure 2**). Respectively, the venous and arterial vWF were 116.4%±14.5% and 117.9%±15.1%. The concentrations of venous and arterial hsCRP were 5.26±0.83 mg/L and 5.19±0.76 mg/L separately. The inner-group comparison also displayed no significant difference on vWF and hsCRP levels in both venous and arterial serum for the treatment group: vWF, venous as 148.3%±16.4% and arterial as 147.6%±17.3%; hsCRP, venous as 7.98±1.02 mg/L and arterial as 7.92±1.27 mg/L. However, comparisons between the control and treatment group demonstrated significant difference on both vWF and hsCRP levels in either venous or arterial samples (**Figure 2**).

To determine instant effects of PLEIs on endothelial function and inflammatory response, site-specific vWF and hsCRP levels in ischemic locations were assessed and compared between pre- and post treatment. Firstly, significantly higher vWF and hsCRP levels were noticed in ischemic sites than that in venous or distal arterial parts (**Figures 2** and **3**). Immediately post PLEIs, the vWF level in ischemic regions was increased from 231.3%±

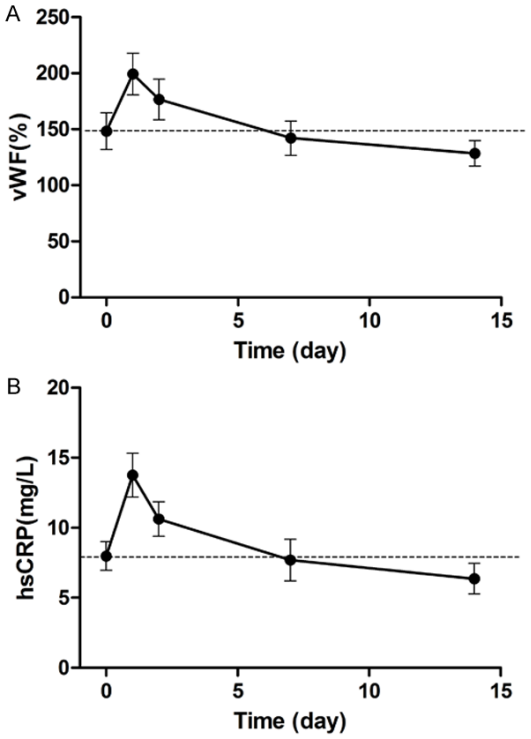

Figure 4. A. Dynamic changes of venous plasma vWF levels in patients with both T2D and PAD post PLEI. B. Dynamic changes of venous plasma hsCRP levels in patients with both T2D and PAD post PLEI.

32.6% to 299.2%±35.2% (**Figure 3A**), which implies endothelial function impairment by the invasive invention. While the hsCRP concentrations in ischemic sites were not remarkably altered by the treatment, with 14.82±2.01 of pre-treatment and 15.08±2.28 of post treatment (**Figure 3B**).

Venous vWF and hsCRP levels were monitored in 2 weeks post PLEIs to reveal the dynamic changes of endothelial function and inflammatory response in treated subjects. Both vWF and hsCRP reached their highest levels in the vein at 24 hours after treatment, levels of which were 199.3%±18.6% and 13.76±1.57 mg/L relatively (**Figure 4**). 48 hours after PLEIs, the venous vWF (176.5%±18.1%) and hsCRP (10.63±1.23 mg/L) were still significantly higher than those of pre-treatment. About 1 week after treatment, the vWF and hsCRP levels were both decreased to the level of pre-treatment. Gradually, 2 weeks after treatment, both of these factors were improved significantly compared to pre-treatment with vWF level as 128.5%±11.4% and hsCRP concentration as 6.36±1.09 mg/L (**Figure 4**).

Discussion

Alterations of endothelial function and inflammatory response by percutaneous interventions have been extensively evaluated in the management of arteriosclerosis, plaque rupture, and especially coronary disease using vWF and hsCRP as markers [14, 21, 22]. However, there is little evidence for vWF and hsCRP assessments in the arterial ischemic sites of patients after percutaneous interventions due to the risk and obstacle of blood collection, such as from coronary artery.

Currently, rapid advances in percutaneous revascularization techniques and equipment for lower-extremity arterial interventions have largely reduced the risk and difficulty of sample collection from the lower-extremity artery. This allows the opportunity to explore effects of PLEIs on vWF and hsCRP levels in ischemic sites and venous blood stream of patients with both T2D and PAD in current study. Hundreds of PLEIs have been successfully applied to patients with both diabetes and lower extremity PAD in the hospital since 2007 [7], where this study was completed.

To provide clues for the effect of PLEIs on endothelial function and inflammatory response in patients with both T2D and PAD, we measured site-specific levels of vWF and hsCRP in ischemic spots and also their global changes in artery and vein blood pre- and post treatment. Prior to PLEIs, there is no significant difference on both vWF and hsCRP levels between arterial and venous blood of the treatment group, suggesting that observations from vein may stand for the global levels of these two factors. Whereas both vWF and hsCRP levels of the treatment group are significantly here than that of the control group, which implies endothelial dysfunction and hyperactive inflammatory response in T2D patients with lower extremity PAD. Plasma levels of vWF and hsCRP in the arterial ischemic regions are both significantly higher than that in venous or distal arterial blood of treatment subjects, which also indicates even worse endothelial dysfunction and stronger inflammatory response in ischemic sites. More severe thrombosis could be caused by further endothelial dysfunction and hyperactive inflammatory response [14, 22], thus it is important to perform early protection of endothelial function and considerations of anti-inflammation as well as anti-clotting. Our site-

specific test on vWF and hsCRP level changes in ischemic regions displays that vWF is instantly increased by PLEIs, meaning immediate endothelial damage brought by the treatment. This result may draw clinic attention on urgent anti-clotting needs at the time of balloon dilation or stent implantation during PLEIs in patients with T2D and PAD. Concentration of hsCRP in ischemic sites is not immediately changed by PLEIs, which may be explained by its slow producing rate [23].

Dynamic changes of vWF and hsCRP levels following PLEIs were monitored for 2 weeks in this study. Venous levels of vWF and hsCRP significantly increase right after PLEIs, reach the highest levels at 24 hours post treatment, and decrease to a lower level than that of pre-treatment in 2 weeks. This result implicates that the endothelial function impairment and activated inflammatory response induced by PLEIs can restore or even be improved with rational management in 2 weeks post treatment. It suggests that the rational care from this study, including endothelial protection, anti-inflammatory response and anti-clotting treatment, could be directly followed or modified in relevant future applications. Due to the limited observation time and number of recruited subjects for this study, some issues remain elusive such as long-term effects of PLEIs on recovery of PAD in patients with both T2D, and the relationship of vWF and hsCRP changes with the number of treated blood vessels.

Disclosure of conflict of interest

None.

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