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Original article

The association of circulating inflammatory and oxidative stress biomarker levels with diagonal earlobe crease in patients with atherosclerotic diseases

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ABSTRACT

Background: Earlobe creases (ELC) are frequently observed in patients with atherosclerosis. Atherosclerosis is considered to be a systemic vascular inflammatory disease, and oxidative stress is known to be a contributor to vascular inflammation. It is well known that inflammatory biomarkers [high-sensitivity C-reactive protein (hs-CRP), pentraxin3 (PTX3)] and oxidative stress markers [malondialdehyde low-density lipoprotein (MDA-LDL)] are associated with atherosclerotic changes. This study was designed to test the hypothesis that biomarkers of inflammation and oxidative stress are increased in patients with ELC.
Methods: A total of 223 consecutive patients with atherosclerotic risk factors were enrolled and divided into two groups. One group consisted of patients with ELC (ELC group, $n = 134$) and the other was without ELC (non-ELC group, $n = 89$). Medical information and biomarker levels related to atherosclerosis were acquired from these patients.
Results: The male ratio, prevalence of hypertension, diabetes mellitus, and MDA-LDL, hs-CRP, and PTX3 levels were found to be higher in the ELC group, compared with the non-ELC group. A multiple logistic regression analysis showed that PTX3 levels, rather than hs-CRP, constituted the strongest predictive factor for the appearance of ELC.
Conclusions: Vascular inflammation and oxidative stress are associated with the presence of ELC.

Introduction

Diagonal earlobe creases (ELC) are frequently observed in patients with coronary artery disease (CAD) [1]. This association was first reported by Frank in 1973 [2], and has been recognized to be a simple cutaneous marker in patients with CAD. Previous studies have provided evidence that carotid artery intima-media thickness is increased in patients with ELC, implying that ELC is closely associated with atherosclerosis [3,4].

It has been proposed that atherosclerotic diseases, including CAD, can be attributed to systemic vascular inflammation induced by oxidative stress [5]. Indeed, inflammatory cell (*e.g.* macrophages) activation and infiltration are frequently observed in impaired vascular walls. Cardiovascular risk factors trigger oxidative stress, which could induce vascular inflammation and promote atherosclerotic changes. Previous studies have provided evidence that high-sensitivity C-reactive protein (hs-CRP) is linked with vascular inflammation and is closely associated with the prognosis of patients with atherosclerotic disease, such as CAD [6]. Moreover, pentraxin3 (PTX3) has emerged as a biomarker for vascular inflammation [7]. Because PTX3 is mostly produced in macrophages, smooth muscle cells, and endothelial cells under stimulation with interleukin-1 or tumor necrosis factor α, PTX3 is known to be elevated in patients with atherosclerosis [7]. Therefore, PTX3 is a more sensitive biomarker for vascular inflammation than CRP. Furthermore, malondialdehyde low-density lipoprotein (MDA-LDL) is a major component of oxidized LDL and is a useful surrogate marker of oxidative stress [8]. Reactive oxygen species such as superoxide and activated radicals lead to the oxidative modification of the LDL. Clinical studies have shown that MDA-LDL is elevated in patients with atherosclerosis [9,10].

Although ELC is closely associated with atherosclerotic changes, there is no evidence indicating an association between ELC and systemic vascular inflammation or oxidative stress. This study was designed to test the hypothesis that the presence of the ELC is associated with vascular inflammation and oxidative stress, which could lead to atherosclerosis.

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Methods

Study population

From January 2013 to December 2013, we enrolled 223 consecutive patients experiencing coronary risk factors (hypertension, diabetes mellitus, and dyslipidemia) in the cardiovascular outpatient clinic at the Akita University Hospital. hs-CRP and PTX3 as inflammatory biomarkers, and MDA-LDL as an oxidative biomarker were added to routine blood sampling. Before receiving the results of the blood tests, the presence of ELC was checked in each patient based on the following diagnostic criteria [3]. Briefly, the earlobe structure was first sketched in sitting position on the patient's chart. Second, if there appeared to be ELC, the characteristics (*e.g.* length, depth, width, and the number of creases) of each earlobe crease were recorded and evaluated. A diagonal ELC is defined as a deep crease that extends from the tragus toward the outer border of the earlobe, covering at least two-thirds of the earlobe length. If an ELC was observed on at least one earlobe, we assigned the patient to the ELC group (Fig. 1). To avoid the examiner's bias, the earlobes were evaluated by other cardiologists who were blinded to the study protocol.

Consequently, enrolled patients were divided into two groups: one group was the ELC group ($n = 134$) and the other was the non-ELC group ($n = 89$). Physical information, cardiovascular risk factors, medical histories, and medication data were compared between the two groups. Patients with infectious diseases, left ventricular contractile dysfunction or systolic heart failure (left ventricular ejection fraction <55%), chronic kidney diseases, obstructive pulmonary diseases, symptomatic previous myocardial infarction, cerebrovascular accident, peripheral artery disease, prior coronary intervention, coronary surgery, and malignant diseases were excluded from this study.

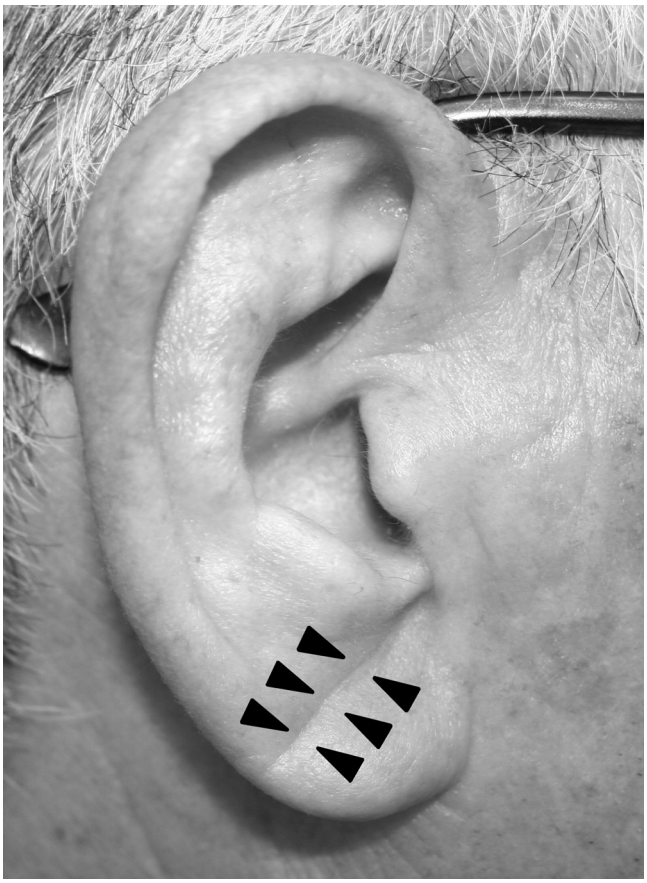

Fig. 1. Typical example of earlobe crease in a patient with atherosclerotic disease.

Definitions of cardiovascular risk factors

Information regarding cardiovascular risk factors (*e.g.* cigarette smoking hypertension, diabetes mellitus, and dyslipidemia) was acquired from the enrolled patients. Additionally, the medical history and current medications of all subjects were recorded. Patients experiencing systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg or receiving anti-hypertensive drugs were diagnosed with hypertension. Diabetes mellitus was diagnosed as a patient with fasting plasma glucose levels ≥126 mg/dl or glycosylated hemoglobin levels ≥6.5% according to the guidelines of the Japan Diabetes Society. Patients using insulin or hypoglycemic drugs were also diagnosed with diabetes mellitus. Dyslipidemia was defined as a fasting LDL level ≥140 mg/dl, triglycerides ≥150 mg/dl, a decreased high-density lipoprotein (HDL) level <40 mg/dl, or current treatment with lipid-lowering drugs. Cigarette smoking signifies a current smoker *vs.* nonsmokers.

Acquisition of inflammatory and oxidative biomarkers

Serum PTX3 levels were measured by enzyme-linked immunosorbent assay (ELISA) using the Human Pentraxin3/TSG-14 ELISA system (Perseus Proteomics Inc., Tokyo, Japan) with an automatic analyzer (Kyowa Medics Co., Ltd., Tokyo, Japan). Serum hs-CRP levels were acquired using an ultra-sensitive latex-enhanced immunoassay (CRP-Latex, Denka Seiken Co., Ltd., Tokyo, Japan) with an automatic analyzer (LABOSPECT008, Hitachi-medical Co., Ltd., Tokyo, Japan). Plasma MDA-LDL levels were also assayed using an ELISA (Kyowa Medics Co., Ltd., Tokyo, Japan) with an automatic analyzer (Sekisui Medical Co., Ltd., Tokyo, Japan).

Statistical analysis

Continuous variables are expressed as the mean ± standard deviation. For continuous and normally distributed data, a Student's *t*-test was used for comparisons between groups. For non-normally distributed data, a Mann–Whitney *U*-test was used. Multiple logistic regression analyses were used to identify the influential parameters on the appearance of diagonal ELC. All parameters with a p value <0.10 in the univariate analyses were entered into the multivariate analysis. Plasma hs-CRP values were natural-log-transformed for the multivariate analysis. A p value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL, USA).

Results

Baseline characteristics of the 223 enrolled patients are summarized in Table 1. Gender ratio (ELC group, 73.9% *vs.* non-ELC group, 53.9%, $p = 0.002$), the prevalence of hypertension (ELC group, 82.8% *vs.* non-ELC group, 62.9%, $p = 0.001$), the ratio of patients with diabetes mellitus (ELC group, 40.3% *vs.* non-ELC group, 19.1%, $p = 0.001$), and the prevalence of coronary artery disease (ELC group, 29.1% *vs.* non-ELC group, 15.7%, $p = 0.022$) were found to be higher in patients with ELC. Medication data were not different between the two groups.

Fig. 2 shows serum hs-CRP levels in both groups. Serum hs-CRP levels were significantly elevated in the ELC group, as compared with the non-ELC group (ELC group, 0.12 ± 0.02 mg/dl *vs.* non-ELC group, 0.03 ± 0.01 mg/dl, $p < 0.001$). The PTX3 levels of the two groups are compared in Fig. 3. PTX3 levels were higher in the ELC group compared with the non-ELC group (ELC group, 3.28 ± 1.45 ng/ml *vs.* non-ELC group, 1.65 ± 0.98 ng/ml, $p < 0.001$, respectively). MDA-LDL levels of