Original Article

The Apolipoprotein B concentration in Gingival Crevicular Fluid of Diabetes Mellitus Patients

Sanjiv Kumar Bansal, Department of Biochemistry, SGT Medical College, Hospital and Research Institute, Gurgaon.

Abstract

Background: Oxidative modification of low-density lipoprotein (LDL) occurs in various diseased tissues and sites of local inflammation. Aim: In this study, we investigated the levels of apolipoprotein B (apoB) and oxidized low-density lipoprotein (oxLDL) in the gingival crevicular fluid (GCF) of DM patients. Materials and Methods: Human gingival crevicular fluid was sampled from healthy gingival sulci (pocket depth < 4 mm, n = 14) of 18 DM patients and 18 healthy subjects were examined. GCF was collected with paper points without inflicting any harm. The apoB and oxLDL levels were measured by sandwich ELISA assays. Results: The GCF volume and the concentrations of protein, apoB and oxLDL in GCF were significantly higher in the DM patients than control subjects. In particular, the apoB concentration in GCF was increased 8-fold in the DM patients. Conclusion: GCF could be a source for evaluating not only the oral status of patients, but also certain systemic conditions.

Keywords: Diabetes mellitus, Gingival crevicular fluid, Apolipoprotein B.

Corresponding Author: Dr. Sanjiv Kumar Bansal, Department of Biochemistry, SGT Medical College, Budhera, Gurgaon, Haryana India. Email:drsanjivbansal@rediffmail.com

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Introduction

Gingival crevicular fluid is exuded from the surrounding tissue of periodontal pockets in response to the pathogenic process in periodontitis. Gingival crevicular fluid contains microbial plaque, host inflammatory cells, host tissue and serum-derived factors. As periodontal disease progresses, the volume of GCF increases and inflammatory cytokines appear in the GCF. GCF can be easily and non-invasively collected using dental paper points, so that it has been studied for clinical examinations.[1] In particular, periodontal disease is considered to be one of the complications of diabetes mellitus (DM). Periodontitis is a chronic infectious disease affecting the tissues surrounding and supporting the teeth, caused by gram-negative bacteria in dental plaque.[2] It is well established that patients with DM have at least a 2-fold increase in the risk of periodontal disease compared with non-diabetic subjects.[2] Epidemiological studies have reported an association between periodontal disease and dyslipidemic conditions.[2] Based on these we can hypothesize that Gingival crevicular fluid is a potentially a good substrate from which to estimate both the conditions of local gingival tissues and the systemic status.

Materials and methods

The subjects of this study were fully informed of the protocol, and their written informed consent was obtained according to the Declaration of Helsinki. Ethical permission was taken from ethical

committee of Institution before the commencement of study. A total of 20 subjects (10 diabetic patients and 10 control subjects) were selected. Patients with systemic disease and pregnancy or lactation in females were excluded from study. All the subjects had healthy gingivae and showed no significant inflammation or attachment loss. Two GCF samples at the sites of two different teeth and a venous blood sample were collected from each participant. The periodontal status such as probing pocket depth (PPD), bleeding on probing (BOP) and the parameters for hyperglycemia and hyperlipidemia were measured. Venous blood (26 mL) sample was taken and evaluated further for HbA1c, glycoalbumin (GA), fasting blood glucose, triglyceride (TG), LDL-cholesterol (LDL-C), and HDL-cholesterol (HDL-C), and malondialdehyde-modified LDL (MDA-LDL) was measured using an ELISA procedure. The chylomicron (CM), very low-density lipoprotein (VLDL) and LDL fractions were separated using sequential ultracentrifugation.

GCF samples were collected by using two buccal maxillary anterior teeth with shallow pockets and stored at 4°C temperature. The concentrations of oxLDL in the GCF and plasma samples were measured using a sandwich ELISA protocol.[6,7] The measurement of apoB was carried out by sandwich ELISA.[8] The apoB values in the LDL, VLDL and CM fractions were combined to estimate the total apoB plasma concentration. Results obtained were statistically analysed using Student's paired t-test and Pearson's correlation with application of Stat-view version 5.0.

Results

Table 1 shows the characteristics of the DM patients (n = 10) and non-DM subjects (n = 10) examined in this study. The levels of HbA1c, GA and fasting blood glucose clearly indicated poor glycemic control in the DM patients. Body mass index (BMI), the smoker/non-smoker ratio, LDL-C and MDA-LDL were all slightly higher in the DM patients, but not significantly. HDL-C was lower and TG was higher in the DM patients, suggesting an association of lipid metabolism with DM. (Table 2)

Table 1: Characteristics of the patients

Parameter	Total
Age (years)	46.5 ± 15.2
Females (%)	50.0
Systolic BP (mmHg)	134.1 ± 14.2
Diastolic BP (mmHg)	80.2 ± 10.6
Pocket depth (mm)	2.8 ± 0.5
Cigarette smokers (%)	30.6
Total cholesterol (mg/dL)	212.4 ± 24.0
LDL-cholesterol (mg/dL)	122.8 ± 22.4
HDL-cholesterol (mg/dL)	60.0 ± 13.1
Triglyceride (mg/dL)	118.4 ± 68.2

Table 2: Concentrations of apolipoprotein B (ApoB) in gingival crevicular fluid and plasma low-density lipoprotein (LDL) Fractions

Patient number	ApoB in gingival crevicular fluid (μg/mL)	Plasma ApoB (mg/mL)
1	48.6	4.42
2	264.6	2.64
3	24.4	4.28
4	52.6	4.66
5	40.6	4.24
6	46.0	3.60
7	187.8	4.28
8	6.2	2.20
9	44.2	2.40
10	24.2	2.80
Average	56.4 ± 62.5	2.17 ± 0.99

Periodontal disease is reportedly the sixth most common complication of DM. GCF samples were collected from two non-diseased teeth per subject; the average PPDs of the GCF collection sites were less than 3 mm (Table 2). Bleeding on probing (BOP) indicates the disease activity at the probing site. The percentages of teeth with deep PPD

or BOP and the number of residual teeth were significantly higher in the DM patients than non-DM subjects (Table 2). BOP correlates positively with HbA1c, GA and fasting blood glucose. Even stronger correlations are observed between the percentage of deep PPD and the DM parameters. The percentage of deep PPD also correlates with TG and MDA-LDL. The GCF from DM patients and healthy subjects shows different characteristics. The volume and protein concentration, apoB and oxLDL concentrations of the collected GCF samples were significantly higher in the DM patients than in the non-DM subjects ($p < 0.01$).

Discussion

In the present study, we found the apoB concentrations to be remarkably higher in the GCF from the DM patients than the healthy subjects. In particular, the GCF apoB concentration exhibited a good correlation with DM as well as the hyperlipidemic parameters.

It is now well established fact that periodontal disease is a complication of DM. The DM patients revealed significant increase in their periodontal scores as well as the DM parameters. To minimize the possible effects of periodontitis on GCF, we collected GCF from non-diseased sites, since GCF from a diseased site could be a reflection of the inflammatory reactions in the gingiva as well as systemic conditions.[3] In addition, although the volume of GCF increases when periodontal disease progresses, collecting GCF from diseased sites eroded by chronic inflammation is often accompanied by bleeding.

Our data show that the GCF volume and protein and oxLDL concentrations were significantly higher in the DM patients than in the healthy subjects. It cannot be denied that a local inflammatory response is also involved in the increase in GCF and the change of the components in GCF. We conjecture that the increased protein concentration and oxLDL level are not caused solely by local inflammatory responses, but rather, there must also be an effect of the systemic disease. The gingival capillaries are enriched in the tissue attached to teeth compared to the intraoral surface of the gingiva.[4] The volume and components of GCF may thus be strongly influenced by endothelial function in the capillaries. Some of pro-inflammatory cytokines, such as IL-1β and IL-8, are increased in the GCF from DM patients that increase vascular permeability.[5] It is well known that the vessels in many different tissues are damaged in DM patients, and increased extravasation of leukocytes and macromolecules under hyperglycemic conditions has been reported for gingival tissue.[6]

ApoB and oxLDL in GCF showed no correlation with either apoB or oxLDL in plasma, suggesting that the permeation of apoB into GCF is independent of the plasma apoB concentration. Also, the oxLDL data confirms our previous report that the oxLDL/apoB ratio in GCF was 17-fold higher than that in plasma,[7] supporting the notion that oxLDL in GCF does not simply originate from plasma. It is noteworthy that only 3 out of 18 DM patients in this study had history of diseases closely related to atherosclerosis, i.e. cardiovascular diseases, cerebral infarction, and atherosclerosis obliterans. In addition, the apoB concentration in the DM patient GCF was 6-times higher than that in healthy subjects, while the GCF protein concentration in the DM patients was only 1.6-times higher than that in healthy subjects. No GCF sample from the healthy subjects had an apoB concentration higher than 250 ng/mL, while the plasma apoB concentration is estimated to be 5 μg/mL when it is diluted in PBS in the same way as GCF samples. It is thus suggested that the release of an extremely large protein-like apoB from plasma into

GCF is tightly regulated. LDL can be transferred from the vessel lumen to the tissue area beyond the endothelial cell layer. Receptor-mediated transcytosis of LDL was demonstrated in endothelial cells from the aorta and brain capillaries in culture[8] and the in vivo evidence for the extravasation of LDL was also reported. It is possible that LDL-receptor function in the gingival capillaries might be affected under DM conditions and thus the apoB concentration increases in GCF. This might also explain why the GCF apoB concentration correlates with the hyperlipidemic parameters in addition to the DM parameters. Further study is certainly needed to clarify the cause of the selective increase of apoB in GCF. The ROC analysis of the GCF apoB concentration suggests that it may be a sensitive marker for hyperglycemic conditions, since the GCF apoB concentration is very stable in healthy people. Recently, a number of studies suggested that periodontitis is associated with the progression of cardiovascular diseases.[9] In contrast, it is indicated that several systemic disorders, such as diabetes, obesity, hypertension and hyperlipidemia, are risk factors for periodontitis. Although an association between periodontitis and lipid metabolism has been reported, the precise biochemical mechanism has not yet been clarified. Some studies have focused on the tissue oxidative stress, examining total oxidant status and lipid peroxidation, leading us to consider the possible occurrence of OxLDL in periodontal tissues.[10]

Conclusion: GCF could be a clinical source to study some systemic conditions, not just the oral status.

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