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Circulating cell-free DNA is associated with cardiometabolic risk factors: The Health 2000 Survey

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ABSTRACT

Cell-free circulating DNA (cf-DNA) has recently arisen as a promising biomarker in acute cardiovascular pathologies and as a mortality predictor in myocardial infarction. We wanted to investigate whether the baseline cf-DNA concentration could serve as an indicator of increased cardiovascular risk and early atherosclerosis. The study population consisted of 1337 participants (aged 46–77 years) in the Health 2000 Survey. cf-DNA was quantified directly in plasma using the fluorescence-based Quant-iT™ high-sensitivity DNA assay kit. Increased cf-DNA levels paralleled a cluster of cardiometabolic risk factors, such as high blood pressure, unfavorable lipid metabolism profile and systemic inflammation in both sexes. In addition, higher cf-DNA levels indicated decreased arterial elasticity and glucose intolerance in women not using hormonal replacement therapy (HRT). The cf-DNA level was also observed to be an independent determinant for Young's elastic modulus but not for carotid artery compliance or beta stiffness index in the women not using HRT. Hence, we conclude that cf-DNA could serve as an auxiliary biomarker in cardiometabolic risk assessment and as an indicator of arterial stiffness in women not using HRT.

1. Introduction

Circulating cell-free DNA (cf-DNA) has been established as a novel marker of cellular death and tissue damage in a variety of acute and chronic pathologies. Elevated cf-DNA levels have been frequently observed in patients with acute cardiovascular disease (CVD), sepsis, trauma, aseptic inflammation and cancer [1,2]. Depending on the condition, both apoptosis and necrosis can serve as the source of the circulating cf-DNA [1]. In patients with myocardial infarction (MI), the cf-DNA levels have been shown to correlate with traditional markers of myocardial damage, including creatine kinase (CK) and cardiac troponins, and to be associated with the post-infarction clinical course [3,4]. The results from two independent studies have also demonstrated that the cf-DNA levels in patients with acute coronary syndrome (ACS) correlate with the severity of the coronary artery lesions [5] and predict the 2-year mortality [6], thereby aiding the risk stratification of ACS. Although other studies did not observed such correlations, the authors nevertheless reported significantly elevated cf-DNA concentrations in MI patients and proposed that cf-DNA assessment could complement CK and troponin in a multimarker test format [7,8].

Despite its promising utility in acute cardiovascular events, the relationship between cf-DNA and the development of atherosclerosis is unknown. That is, whether the cf-DNA assessment shows utility in the subclinical phases of atherosclerosis or its usefulness is restricted to acute CVD is unresolved. We previously observed that increased cf-DNA levels indicated systemic inflammation and frailty in elderly individuals [9,10]. Therefore, considering that the pathogenesis of atherosclerosis is accompanied by tissue damage

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and systemic inflammation, we wished to address whether circulating cf-DNA concentrations parallel the cardiometabolic risk factors and early vascular changes. The current study material also allowed us to evaluate whether hormone replacement therapy (HRT) modulated the value of cf-DNA as a potential cardiovascular risk marker in women.

2. Materials and methods

The participants in this study ($n = 1337$; 682 men and 655 women, aged from 46 to 77 years) consisted of a supplementary sub-cohort of the Health 2000 Survey—a large cross-sectional health examination survey conducted in 2000 and 2001. The Health 2000 Survey participants represent the general Finnish population aged 30 years and older. The overall study cohort was a two-stage stratified cluster sample consisting of 8028 persons (for details of the study design, see Ref. [11]). The data in the current study involve the individuals who had a complete carotid ultrasound data and information about HRT use (women) available. The study protocol of the Health 2000 Survey has been approved by the Epidemiology Ethics Committee of the Helsinki and Uusimaa Hospital region. The study was performed following the guidelines of the Declaration of Helsinki and all the participants gave their written informed consent.

Venous blood was drawn after an overnight fast. The determination of the plasma lipid levels was performed as previously described [12]. The plasma cf-DNA quantity was measured using a Quant-iT™ DNA High-Sensitivity Assay kit and a Qubit® fluorometer (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. At the mean cf-DNA level of 0.625 μg/ml, the intra- and inter-day variation coefficients for the Quant-iT™ DNA High-Sensitivity Assay were 3.35% and 3.32%, respectively, and at the mean level of 1.585 μg/ml, the corresponding values were 2.67% and 2.50%, respectively. The plasma concentrations of insulin, glucose, C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) were determined as previously described [13]. The homeostasis assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5. Blood pressure and pulse pressure were assessed as previously described [14]. Data about the subjects' smoking history were obtained from the Health 2000 Survey questionnaire.

For the assessment of the carotid artery intima-media thickness (IMT) and elasticity indices, a high-resolution B-mode carotid ultrasound examination of the right carotid artery was performed using a 7.5 MHz linear array transducer as previously described [12]. Three different indices of arterial elasticity, carotid artery compliance (CAC), beta stiffness index (SI) and Young's elastic modulus (YEM) were determined [12] to obtain comprehensive information about arterial stiffness.

The statistical analyses were performed using the IBM SPSS Statistics version 19 (IBM Corp., Sommers, NY, USA). The differences in the cf-DNA distribution between men and women and between women on and not on HRT were assessed with the Mann–Whitney *U*-test. Correlations between the cf-DNA levels and the study variables were analyzed using either Spearman's rho or Pearson's rho (with log-transformed cf-DNA values) when appropriate. Stepwise multivariate linear regression models were used to assess the relationship between the cf-DNA levels and the indices of arterial elasticity (CAC, SI and YEM) in women not using HRT. For this purpose, all variables with a skewed distribution were log-transformed. Variables entered into each of the regression models were selected based on their association with the given elasticity index in univariate testing; variables demonstrating a statistically significant association of $p < 0.005$ were included. In addition, multicollinearity diagnostics were performed to identify and exclude strongly interdependent variables from the models. The included variables were the following: age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), high density lipoprotein (HDL)-cholesterol level, low density lipoprotein (LDL)-cholesterol level, triglyceride level, glucose level, insulin level, cf-DNA level, CRP level, IL-6 level and TNF-α level for CAC; age, SBP, DBP, pulse pressure, LDL-cholesterol level, total cholesterol level, triglyceride level, glucose level, insulin level, cf-DNA level, CRP level, IL-6 level and TNF-α level for SI; and age, BMI, SBP, DBP, LDL cholesterol level, total cholesterol level, triglyceride level, glucose level, insulin level, cf-DNA level, CRP level, IL-6 level and TNF-α level for YEM.

3. Results

The characteristics of the study population are presented in Table 1. Men had significantly higher cf-DNA levels than women not using HRT (0.88 vs. 0.83 μg/ml, $p < 0.00$, Table 1), and the women using either of the HRT regimens, which were estrogen-only preparation (E) or an estrogen–progestin combination (E + P), had significantly lower cf-DNA levels compared with the women not using HRT (0.77 μg/ml vs. 0.83, $p < 0.001$, Table 1). The correlates for the cf-DNA levels are presented in Table 2. Linear regression models assessing the relationship between the cf-DNA level and arterial stiffness indices in women not using HRT are presented in Table 3.

4. Discussion

The results of this study demonstrate for the first time that circulating cf-DNA levels are associated with various cardiovascular risk factors in clinically healthy individuals. The assessment of the correlates for the cf-DNA levels in women according to the HRT use status revealed an interesting pattern. The most prominent associations were observed in women not using HRT: increased cf-DNA levels indicated arterial stiffness (higher SI and YEM and lower CAC), systemic inflammation (elevated CRP, IL-6 and TNF-α levels), impaired glucose metabolism (higher HOMA-IR and insulin levels) and elevated blood pressure. However, in women on either type of HRT, most of the associations were attenuated, indicating that current HRT use overrides the propensity of cf-DNA to reflect increased cardiometabolic risk. Alternatively, because perimenopausal HRT has been reported to ameliorate the CVD risk profile with regard to insulin resistance, endothelial cell function and lipid levels [15], the variation of the cf-DNA concentrations in HRT-using women may not be connected to atherosclerosis-related tissue damage and cell death. In effect, our observation that the HRT-using women have lower cf-DNA levels than non-users supports this hypothesis. Nevertheless, given that the evidence for HRT-mediated cardiovascular protection is still controversial [15] and that we did observe a direct correlation between the cf-DNA levels and inflammation in HRT-using women, this issue warrants further investigation.

With regard to the women not using HRT, further examination into the relationship between the cf-DNA levels and arterial stiffness revealed that the cf-DNA level remained an independent determinant only for YEM in the linear regression models. For CAC and SI, the univariate associations of the cf-DNA level were attenuated to the null when other variables, such as age, blood pressures and LDL-cholesterol, were added to the models. Because YEM is an IMT-independent measure of arterial wall stiffness [16], the cf-DNA level seems to be most tightly connected to the intrinsic arterial elasticity characteristics. Considering that none of the conventional inflammatory markers (CRP, IL-6 and TNF-α) appeared to be determinants for YEM (or the other elasticity indices), the plasma cf-DNA level may capture and reflect a unique aspect of inflammation-