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Leukocyte telomere length and coronary artery calcification in Palestinians

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ABSTRACT

Objective: Shorter leukocyte telomere length (LTL) is associated with higher incidence of coronary heart disease (CHD) and increased mortality. We examined the association of LTL with coronary artery calcification (CAC), which reflects the cumulative burden of coronary atherosclerosis, in an urban Arab sample of Palestinians, a population at high risk of CHD.
Methods: Using a cross-sectional design, a random sample of East Jerusalem residents, comprising 250 men aged 45–77 and women aged 55–76 and free of CHD or past stroke, was drawn from the Israel national population register. LTL was measured by Southern blots. CAC was determined by 16-slice multidetector helical CT scanning using Agatston scoring. We applied multivariable logistic modeling to examine the association between sex-specific tertiles of LTL and CAC (comparing scores >100 vs. <100, and the upper third vs. the lower 2 thirds), controlling for age, sex, education and coronary risk factors.
Results: CAC, evident in 65% of men and 52% of women, was strongly associated with age (sex-adjusted Spearman's rho 0.495). The multivariable-adjusted odds ratios for CAC >100 (found in 30% of men and 29% of women) were 2.92 (95% CI 1.28–6.68) and 2.29 (0.99–5.30) for the lower and mid-tertiles of LTL vs. the upper tertile, respectively ($P_{trend} = 0.008$). Findings were similar for CAC scores in the upper tertile ($P_{trend} = 0.006$), and persisted after the exclusion of patients with diabetes or receiving statins.
Conclusions: Shorter LTL was associated with a greater prevalence of asymptomatic coronary atherosclerosis in an urban Arab population-based sample. Mechanisms underlying this association should be sought.

1. Introduction

Shorter leukocyte telomere length (LTL) has been shown to be associated with a higher incidence of coronary heart disease (CHD) (e.g. [1,2]) and with increased mortality [3,4]. With regard to the association of LTL with underlying atherosclerosis, LTL has been inconsistently related to carotid intima-media thickness (C-IMT) [2,5,6]. Coronary artery calcification (CAC), in contrast with C-IMT, predicts CHD independently of the traditional risk factors (e.g. [7,8]), and was the strongest predictor of CHD risk among a series of "newer" CHD risk markers evaluated in the prospective Rotterdam study of people initially free of manifest CHD. In contrast, C-IMT did not play an independent role [9]. CAC is indicative of the extent of the

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coronary atherosclerosis burden [10] and as such is a useful subclinical measure of coronary artery disease. Of two studies in young to middle-aged adults that examined the association of LTL with CAC, one showed a strong cross-sectional association of LTL with the presence and extent of CAC in men and women aged 40–64 [11]. The second reported, in a small sample of 129 men aged 33–45, that shorter LTL predicted greater CAC progression in the presence of high leukocyte telomerase activity, but the reverse when telomerase activity was low, with no protective association evident for long LTL [12]. These findings appear to conflict with those of Mainous et al. [11]. The mechanisms underlying a LTL-atherosclerosis association are unknown, and it is debated whether the association is causal. Competing hypotheses have attempted to mechanistically link LTL with atherosclerosis. A noncausal hypothesis posits that the rate of LTL attrition in adulthood is an index of progression of atherosclerosis, because both the pace of atherosclerosis and the rate of LTL attrition are increased by chronic inflammation and oxidative stress. If so, LTL dynamics according to the conventional view serve as a record of the vascular injury that marks atherosclerosis. A competing hypothesis proposes that telomere length endowment at birth and its attrition during childhood are the main determinants of LTL, which reflects the hematopoietic stem cell telomere reserves and their ability to engage in vascular repair. In this context, LTL attrition in adulthood might also play a contributory role in driving TL below a critical level [13,14].

Palestinians have undergone a rapid epidemiologic transition over the past century, and particularly so in the last 50 years, with heart disease, cerebrovascular disease and cancer now the three leading causes of death [15]. CHD incidence in East Jerusalem Palestinians ranked particularly high in standardized international comparisons with the World Health Organization MONICA program [16]. CHD incidence and mortality in East Jerusalem Palestinians were also high compared with the Jewish residents of Jerusalem [16,17]. Although the underlying determinants of these findings are unknown, the high prevalence of the metabolic syndrome and diabetes in Palestinians [15,18] may play a role.

It is within this context that we examined the cross-sectional association of LTL with CAC in a population-based sample randomly drawn from middle-aged and elderly Palestinian residents of East Jerusalem.

2. Methods

2.1. Study design

We undertook a cross-sectional, comparative population-based investigation of Palestinian and Israeli residents of Jerusalem. The study's main goal was to identify differences between these two populations that might explain the high risk of CHD evident in standardized international comparisons among the Palestinians. The sampling methods of the Jerusalem Palestinian-Israeli Risk Factor Study have been reported [19]. Here we focus on the Palestinian arm of the study, as LTL determinations were not made for the Israeli sample.

In brief, following the Six-Day War in 1967 Arab residents of East Jerusalem were accorded the legal status of permanent residents of Israel, with access to the job market, social security and health insurance, and are recorded in the Israel national population registry. An age-sex-stratified random sample of 2000 Palestinian residents from East Jerusalem aged 25–74 (comprising 200 men and 200 women in each 10-year age band, 25–34, 35–44…) was drawn from the population registry. Participants were recruited between 2005 and 2008 by a letter of invitation and follow-up phone calls. Ineligibility criteria included inability to provide informed consent, being institutionalized, housebound, pregnant or within 3 months of giving birth, and having a serious health disorder (such as metastatic cancer or end-stage renal disease). Of the Palestinian sample 29.5% could not be located, and of those located 89.6% were eligible. The 970 participants represented a response rate from all located eligible residents of 76.7%.

The examination comprised a face-to-face interview in Arabic and clinical measurements using standardized methods. Blood samples were collected after a 12-h fast, and CAC was measured at a separate session usually within several weeks.

Eligible for the CT imaging were Palestinian men aged $\geq$45 years and women aged $\geq$55 years who had participated in the initial examination, were free of clinical manifestations of CHD and had a BMI of $<$38 kg/m^2 for women and $<$45 kg/m^2 for men (thus excluding 29 women and one man who exceeded these BMI limits as they would have required a higher radiation dose in the CT procedure than acceptable for healthy volunteers). By December 31, 2007 when we ceased CT examination for logistic reasons, 259 of the 362 eligible Palestinians had been scanned (72%), 14% had refused and 14% were being processed. A CT reading was not available for 1 subject and 8 had missing measures of LTL, leaving 250 individuals for analysis.

All participants provided signed informed consent. The study was authorized by the St Joseph Hospital, the Hadassah-Hebrew University Medical Center, and the Israel Ministry of Health Ethics (Helsinki) Committees. Participants were referred to their physicians with their CAC results and interpretation.

2.2. CT imaging for coronary calcification

2.2.1. Image acquisition

The CT imaging and interpretation were done in 2005–2007 by the Hadassah Imaging (Radiology) Department on the Ein Kerem campus. Images were obtained with a 16-slice Philips *Brilliance* MX 8000 IDT multidetector helical CT scanner. The heart was scanned from the base of the heart to the apex at 3 mm collimation with rotation time of 0.5 s during a single breath-hold. No contrast material was used. The field-of-view was 25 cm. Images were acquired in a low-dose setting [20] adjusted according to BMI levels (Shemesh J, personal communication 2012, and Shemesh J, Evron R, Konen E et al. Measurement of coronary calcium by adapting mAs to body mass index: Prospective validation and reproducibility. Abstract SSA03-06. Radiological Society of North America 90th Scientific assembly, Nov 2004, Chicago, IL) so that exposures for men ranged from 55 mAs (for BMI $<$ 34 kg/m^2) to 88 mAs (for BMI $>$ 42 kg/m^2) and for women between 55 mAs (for BMI $<$ 28 kg/m^2) to 98 mAs (for BMI $>$ 35 kg/m^2). Total scan acquisition time was 20–30 s, depending on patient size. Axial prospective ECG gating was used at 75% of the R–R interval.

2.2.2. Reading and scoring

The cardiac CT series was transferred to a Philips MXVIEW workstation and evaluated using Heart-Beat CS software (Philips, Haifa). The operator, a qualified radiologist, manually placed Regions of Interest (ROIs) around selected areas within a sequence of images. Philips CAC scoring software computed the calcification scores by performing automatic calculation on CT numbers inside these ROIs. The calcification quantification included extracting intensity histograms for each selected region, computing scores per region and combining region scores into composite scores using slice-thickness weighting. Agatston calcification scores were calculated using a threshold of 130 Hounsfield Units.

2.3. Leukocyte telomere length measurement

Buffy coat samples had been stored at $-80\,^{\circ}$C for 4–6 years prior to shipment to Aviv's laboratory in Newark, New Jersey. DNA was