Thoracoabdominal Calcifications Predict Cardiovascular Disease Mortality in Type 2 Diabetic and Nondiabetic Subjects: 18-Year Follow-Up Study

Short running title: Calcifications predict cardiovascular mortality

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Objective- To evaluate cardiovascular disease (CVD) and total mortality associated with thoracoabdominal calcifications.

Research Design and Methods- Thoracoabdominal calcifications of native radiograms were evaluated in 833 subjects with type 2 diabetes and 1292 subjects without diabetes, aged from 45 to 64 years, without prior evidence of cardiovascular disease (CVD). The type 2 diabetic and nondiabetic study cohorts were followed-up for 18 years.

Results- After adjustment for conventional risk factors, marked thoracoabdominal calcifications predicted CVD/total mortality with HR(95% CI) of 1.5(0.8-3.0)/1.8(1.1-2.9) in type 2 diabetic men, 3.0(1.6-5.7)/3.1(1.9-5.0) in type 2 diabetic women, 5.0(2.2-12)/4.0(2.2-7.4) in nondiabetic men, and 7.8(1.8-34)/4.0(1.3-7.0) in nondiabetic women, and in the presence of C-reactive protein below/over 3 mg/l with HR of 2.4(1.3-4.4)/3.0(1.4-6.1) in type 2 diabetic subjects and 4.0(1.5-10.8)/6.6(2.7-16.0) in nondiabetic subjects.

Conclusions- Thoracoabdominal calcifications in native radiograms are significant predictors of CVD and total mortality especially in type 2 diabetic and non-diabetic women with elevated hs-CRP level.
Vascular calcification is initiated by metabolic, mechanical, infectious, or inflammatory injury to vasculature. Its progression is mainly determined by inflammatory response to vascular injury (1). It may precede cardiovascular disease (CVD) morbidity and mortality by years or decades in subjects with type 2 diabetes (2) and in general population (3-6). Medial calcification has been associated with CVD morbidity and mortality in diabetic subjects (7) and in subjects with end-stage renal disease (8). Calcifications can be divided into intimal type, medial type of arterial calcification, cardiac valve calcification, and vascular calciphylaxis (9). These four entities of calcifications are consequences of distinct but overlapping pathophysiological mechanisms which can occur simultaneously. Calcifications may function as a limiting factor for intimal plaque growth and represent a biological response to this process (10). A new perspective to the question of clinical significance of calcification has evolved from the practical need to evaluate CVD effects of medications targeted to bone formation, and the bone density effects of medications targeted to vascular welfare (11,12).

Although inflammation is involved in the initiation and progression of vascular calcification, inflammation and calcification may reflect partly independent processes. A combination of markers of calcification and inflammation might therefore be a good predictor CVD mortality. This study evaluates thoracoabdominal calcifications, and their combination with elevated high-sensitivity C-reactive protein (hs-CRP), in prediction of CVD mortality in a cohort of 2 diabetic and nondiabetic subjects without prior evidence of CVD during an 18-year follow-up.

RESEARCH DESIGN AND METHODS
A detailed description of the original study population including 1059 type 2 diabetic and 1373 nondiabetic subjects aged from 45 to 64 years has been published previously (13). Subjects with prior CVD (n=301) or missing information on calcification (n=6) were excluded. Of these 2125 participants, 1862 subjects (317 men and 370 women with type 2 diabetes, 511 nondiabetic men, 610 nondiabetic women) were available for the analyses of hs-CRP. Endpoints were total and CVD mortality by the ICD-9 codes 390-459. The 18-year follow-up lasted until January 1, 2001.

Hs-CRP was analyzed with latex turbidimetric immunoassay (Wako Chemicals, Neuss, Germany), with detection limit of 0.06 mg/l. The interassay coefficient of variation was 3.3% and 2.7% at mean hs-CRP levels of 1.52 (n=116) and 2.51 (n=168) mg/l. From lateral lumbar radiograms, taken with patient in a standing position, thoracoabdominal calcification was graded by a radiologist (M.S.) from no calcification (grade 0) to marked calcification (grade 3) blinded to clinical data. The κ coefficient (SE) for intra-observer variation was 0.87 (0.24).

Data analyses were conducted with the SPSS 14.0.1 program (SPSS, Chicago, IL). Cox models for CVD and total mortality were adjusted for age, gender, status of diabetes, area of residence, current smoking, systolic blood pressure, total cholesterol, HDL cholesterol, BMI, glomerular filtration rate, urinary protein (log.), fasting glucose (in nondiabetic subjects) or glycated haemoglobin A1 (in type 2 diabetic subjects), and duration of
diabetes (in type 2 diabetic subjects). A P-value less than 0.05 was considered statistically significant.
The Ethics Committees of the Kuopio University Hospital and the Turku University Central Hospital approved the study. All study participants gave informed consent.

RESULTS
During the 18-year follow-up 817 all-cause deaths and 465 CVD deaths occurred. Baseline characteristics are given in Online-Appendix Table A1 which is available at http://care.diabetesjournals.org.
Thoracoabdominal calcifications were more frequent in men than women (66.1 vs. 55.7%), and in type 2 diabetic than nondiabetic subjects (71.5 vs. 53.6%).
The presence of thoracoabdominal calcifications was associated with the presence hs-CRP >3 mg/l in type 2 diabetic women (P=0.006), not quite in nondiabetic women (P=0.069), and not in nondiabetic men (P=0.739) or in type 2 diabetic men (P=0.550).
In multivariate analysis, thoracoabdominal calcifications predicted CVD mortality independently of other risk factors in type 2 diabetic women, nondiabetic men, and nondiabetic women, but not in type 2 diabetic men (Online-Appendix Table A2). CVD survival was markedly dependent on the presence of calcifications and the status of diabetes at baseline, as presented by gender in Online-Appendix FigureA1. There was no interaction between gender and thoracoabdominal calcifications (P=0.678) in prediction of CVD mortality. However, an interaction was observed between gender and hs-CRP in association with thoracoabdominal calcifications (P=0.037 for the interaction term of hs-CRP by gender).
The association of thoracoabdominal calcification grade with CVD and total mortality was consistent in subgroups with hs-CRP <3 mg/l and ≥3 mg/l (Online-Appendix Figure A2). Multivariate Cox analysis, including both hs-CRP and thoracoabdominal calcifications, suggested that they are independent predictors of CVD and total mortality. HR of CVD death for calcification tended to be higher in the presence of elevated hs-CRP through calcification categories from 0 to 3 (Figure 1).

CONCLUSIONS
In this 18-year follow-up study marked thoracoabdominal calcifications predicted CVD and total mortality especially in nondiabetic and type 2 diabetic women with elevated hs-CRP level.
The relative risk associated to calcifications was higher in nondiabetic subjects compared to diabetic subjects and in women compared to men. Predominance of aortic calcification has been observed in women in the Reykjavik study, but gender differences in the risk associated to calcifications have not been observed (14). In our study marked calcifications tended to signify a higher relative risk for CVD mortality in women than in men, but a statistically significant interaction with gender was not observed. The risk increase of CVD-death associated with the presence of calcifications was lower in the presence of low hs-CRP, and higher in the presence of elevated hs-CRP. Calcium deposits may alter or stimulate the process of CVD (1), as they accumulate in dying cells and lipoprotein deposits, and activate endochondral and intramembranous types of ossification by secretion of inflammatory cytokines, tumor necrosis factor–α, interleukin-1 and interleukin-8 (15). In this study, an
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interaction with gender between inflammatory activity and grade of calcifications was observed. Combining hs-CRP - a surrogate of activity of atherosclerosis - with the presence of calcifications - a surrogate of stage of atherosclerosis - the prognostic value improves. Based on the findings of the present study, detection of thoracoabdominal radiological calcification should lead to a detailed evaluation and treatment of CVD risk.

FIGURE LEGENDS

Figure 1. Hazard ratio (95% CI) in Cox multivariate model of cardiovascular disease mortality during 18-year follow-up for grade 1 (slight), grade 2 (moderate), grade 3 (marked) calcification of thoracoabdominal aorta. The reference with HR 1 are the subjects with no thoracoabdominal calcifications (grade 0) and high-sensitivity C-reactive protein (hs-CRP) <3 mg/l. Squares mark subjects with hs-CRP <3 mg/l, and triangles mark subjects with hs-CRP ≥3 mg/l. In multivariate analysis, marked thoracoabdominal calcifications predicted CVD in the presence of high-sensitivity C-reactive below/over mg/l with HR (95% CI) of 2.4 (1.3-4.4) / 3.0 (1.4-6.1) in type 2 diabetic subjects and 4.0 (1.5-10.8) / 6.6 (2.7-16.0) in nondiabetic subjects.
REFERENCES

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Hazard ratio for CVD mortality (95% CI)

Type 2 Diabetic Subjects

Nondiabetic Subjects