Telomere shortening and oxidative stress in abdominal aortic aneurysm and varicose vein: comparing two dilative vascular pathologies

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Oxidative stress and DNA damage
The telomeres...

**Telomeres**
Telomeres are structures on the tips of all chromosomes which gradually get shorter with age. Short telomeres are linked with premature ageing and many diseases. By measuring telomere length, scientists can see how fast someone is ageing and calculate their biological age. This data can then be used to predict life expectancy.

**How the Telomeres Shorten**

- **Cell Division Over Time**
- **Telomeres Shorten with Age**
  - With each cell division, the telomeres are shortened - a sign of ageing - until eventually they are worn away. The chromosomes are then damaged and the cell dies.
Telomere shortening and vascular diseases

- Telomerase
- Longevity genes
- Insulin-like growth factor I
- hSIRT1
- Oestrogen
- Antioxidants

Telomere shortening

- Inhibition
- Induction

Telomere dysfunction

- Genetic factors
- Cell division
- DNA damage and response
- Oxidative stress
- Inflammation
- Ultraviolet irradiation

- Nontelomeric DNA damage

Normal mitotic cell

- Mitogenic signals e.g. active oncogenes
- Nongenotoxic stress, e.g. chromatic changes

- p53
- p21Cip1

- Chk2 and other pathways

- p16ink4a

- Altered cell morphology
- Mitotic arrest

Senescent cell

- Markers of senescence β-Galactosidase

Atherosclerosis
- Coronary artery disease

Senescence-associated secretory phenotype
- Growth factors, IL-1 and IL-8, matrix metalloproteinase, plasminogen activator inhibitor 1, vascular endothelial growth factor, proliferation, inflammation, thrombosis, angiogenesis, and apoptosis

Other cardiovascular pathologies
Our study...

Telomere length in AAA patients?

The study included
-30 AAA patients (mean age 71.3 (range 61–78 years))
-30 apparently healthy control subjects (mean age 68.7 (range 62–77 years)).

Exclusion criteria: neoplasms, infections and chronic inflammation, chronic obstructive pulmonary disease, diabetes mellitus, nephropathy, liver disease, symptomatic obstructive coronary, cerebrovascular diseases, and smoking habits.

1) Blood lymphocytes from peripheral venous blood sample of the superficial vein of the arm.
2) AAA samples from the aneurismal sac at the time of the surgical repair.
3) Skin biopsies at the time of surgery
Telomere length of EC and VSMC from patients with AAA measured using Q-FISH and immunofluorescence.

A) Aortic aneurysmatic wall derived EC stained with anti-CD31 mAb (green). The inset shows the nuclei analyzed by Q-FISH. B) EC interphase nuclei hybridized with Cy3-PNA telomeric probe (red signals). C) Aortic aneurysmatic wall derived VSMC stained with anti-α-smooth muscle actin mAb (green). D) VSMC nuclei hybridized with Cy3-PNA telomeric probe (red signals). DAPI was used to label nuclei.

Telomeres were significantly shorter in EC, VSMC, epidermal cells and peripheral blood lymphocytes from AAA than from normal aorta.

Telomere length of EC, VSMC, blood lymphocytes and epidermal cells from patients with AAA and controls.

A) Telomeres length in EC from AAA patients and in EC from normal aorta. B) Telomeres length in VSMC from AAA patients and in VSMC from normal aorta. C) Telomeres length in epidermal cells from patients with AAA and in these same cells from controls. D) Telomeres length in peripheral blood lymphocytes in AAA patients and in controls.

The percentage of 8-oxo-dG+ nuclei in EC, VSMC, epidermal cells and peripheral blood lymphocytes was significantly augmented in AAA patients compared to controls.

**Oxidative DNA damage of EC, VSMC, blood lymphocytes and epidermal cells from patients with AAA and controls.**

A) The percentage of 8-oxo-dG+ nuclei in EC from AAA patients and in EC from normal aorta. B) The percentage of 8-oxo-dG+ nuclei in VSMC from AAA patients compared to VSMC from normal aorta. C) 8-oxo-dG+ nuclei in epidermal cells from AAA patients and in the same cell type from controls. D) The percentage of 8-oxo-dG+ nuclei in peripheral blood lymphocytes from AAA patients compared to controls.

Inverse relationship between telomere shortening and DNA damage in blood lymphocytes from AAA patients.

Relationship between telomere shortening and DNA damage in blood lymphocytes from AAA patients.

Spearman's correlation test. Linear regression analysis between telomere length and accumulation of ROS-induced oxidative DNA damage, assessed by 8-oxo-dG staining, in blood lymphocytes from patients and from controls. The Spearman's rank correlation coefficient ($r_S$) is $-0.57$.

The mean concentration malondialdehyde (MDA) in plasma from patients with AAA was significantly higher than from controls.

Oxidative stress in plasma from AAA patients and controls. Oxidative stress was evaluated by measuring the MDA concentration by thiobarbituric acid reactive substance/s (TBARS) assay. MDA concentration in plasma from AAA patients and healthy controls

Our study...

Telomere length in varicose vein (VV) patients?

The study included
-10 VV patients (mean age 71.3 (range 61–78 years))
-10 apparently healthy control subjects (mean age 68.7 (range 62–77 years)).

Exclusion criteria: neoplasms, infections and chronic inflammation, chronic obstructive pulmonary disease, diabetes mellitus, nephropathy, liver disease, symptomatic obstructive coronary, cerebrovascular diseases, and smoking habits.

1) Blood lymphocytes from peripheral venous blood sample of the superficial vein of the arm.
2) VV samples at the time of the surgical repair.
Telomere length in ECs from VV patients was similar to that measured in ECs from AAA subjects. Telomeres of peripheral blood lymphocytes were significantly shorter in AAA patients than in VV patients and controls. Lymphocytes from VV patients and from controls showed a similar telomere length.

The telomere length of endothelial cells (ECs) and blood lymphocytes from VV and AAA patients and controls. TL was measured using quantitative fluorescence in situ hybridization (Q-FISH) and fluorescence intensities were expressed in arbitrary telomere fluorescence units (TFU). (A) TL in ECs from VV patients and AAA patients. (B) TL in blood lymphocytes from VV patients, AAA patients and healthy controls. (C) TL in epidermal cells from VV patients and AAA patients.

At the tissue level malondialdehyde (MDA) concentration in VV patients was similar to the AAA group. Conversely, the mean concentration of MDA in plasma from patients with VV was significantly lower than from the AAA group. Moreover, compared to control subjects, VV samples also showed a slight increase of the MDA level.

Oxidative stress in plasma and vascular tissue from VV and AAA patients and controls. Oxidative stress was evaluated by measuring the MDA concentration by thiobarbituric acid reactive substance/s (TBARS) assay. (A) MDA concentration in tissue homogenate from VV patients and AAA patients. (B) MDA concentration in plasma from VV patients, AAA patients and healthy controls.
Inverse relationship between blood lymphocyte Telomere length and plasma oxidative stress in VV patients, AAA patients and controls. Interestingly, the correlation was strong when the statistical analysis was restricted to samples with a MDA level higher than 3.5 nmol/mL, corresponding to the MDA median value of the VV group.

Linear regression analysis and Spearman’s correlation test were performed between the blood lymphocyte TL and MDA plasma concentration in VV patients, AAA patients and healthy controls. Spearman’s rank correlation coefficient was $-0.4034$. On the right, linear regression analysis was performed including only subjects with plasma MDA concentrations higher than 3.5 nmol/mL (corresponding to the median value of the VV group). Spearman’s rank correlation coefficient was $-0.6821$.

Conclusion:

1. EC, VSMC, keratinocytes and circulating blood lymphocytes from AAA patients have shortened telomeres and oxidative DNA damage.

2. At circulating level, serum MDA concentration (oxidative stress marker) was higher in AAA patients than in controls.

Role of local and systemic high oxidative stress as one of the key factors of AAA development

Telomere shortening and oxidative DNA damage occurring in different cellular types suggest the **systemic nature of the disease**
1. At tissue level, VV patients show telomere shortening and high oxidative stress, measured as MDA level, similar to AAA patients.

2. At circulating level VV patients have:
   - Telomere length of blood lymphocytes longer than AAA patients and similar to controls.
   - Oxidative stress measured as MDA serum level significantly lower than AAA patients but higher than controls.

3. Statistical analysis suggests that telomere shortening and oxidative stress are significantly associated, both for AAA and VV patients, for higher levels of oxidative stress.

Key role for local microenvironment conditions strictly associated with high local oxidative stress as one of the factors involved in mediating the alteration observed in VV wall as well as telomere attrition.
Thanks for your attention