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Assessment of candidate ocular biomarkers of ageing in a South African adult population: Relationship with chronological age and systemic biomarkers☆

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ABSTRACT

Certain anatomic and functional parameters of the eye change with increasing chronological age. They may, therefore, serve as potential biomarkers of ageing. We investigated associations between four such ocular parameters (lens density, retinal vessel calibre, corneal endothelial cells and retinal nerve fibre layer thickness) and two 'cellular' biomarkers of ageing (leukocyte telomere length and CDKN2A expression), with frailty (a clinical correlate of biological ageing) in a population of South African adults. All ocular parameters revealed an association with either telomere length or CDKN2A expression. However, lens density was most strongly correlated with age, increased CDKN2A expression, and with frailty ($p = 0.05$ and 0.03, respectively). Narrow retinal arteriolar diameter, associated with increased chronological age, was also associated with increased CDK2NA expression (0.42 vs. 0.31, $p = 0.02$) but not with frailty. Ocular parameters may aid in determining biological age, warranting investigation in longitudinal studies.

1. Introduction

There is substantial variation in the health and functional status of older populations in many developing countries as well as in developed countries (Lloyd-Sherlock et al., 2012). The reasons for these variations are poorly understood, highlighting the need for translational age-related research within a global context (Salomon et al., 2013; Wang et al., 2013). Chronological age is an imprecise measure of biological ageing, due to inter-individual differences in rates of ageing. The disconnection between chronological age and lifespan has led to a search for effective and validated biomarkers of ageing (BoA), defined as "biological parameters of an organism that either alone or in some multivariate composite will better predict functional capability at some late age, than will chronological age" (Baker and Sprott, 1988).

It is acknowledged that many age-related chronic diseases such as cardiovascular disease and Alzheimer's disease share common pathways of early dysregulation, and that the development of markers and diagnostic techniques is fundamental to understanding healthy biological ageing and thus these diseases (Franco et al., 2007). The need for research on how healthy ageing can be achieved in the context of life-time trajectories has led to concept of the 'Healthy Ageing Phenotype' (Franco et al., 2009). With demonstrable molecular, epigenetic and clinical correlates of ageing, the eye may be a model system for validating potential biomarkers (Pathai et al., 2013b).

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Table 1
Biomarkers of aging, methods of measurement and the impact of aging.

Anatomical site	Parameter	Method of measurement	Age-related changes
Peripheral blood leukocytes	Telomere length (TL) CDKN2A expression	qPCR qRT-PCR to estimate mRNA levels	TL shortens Increased expression
Corneal endothelium	Endothelial cell density (ECD) Coefficient of variation (CV) Hexagonality index (Ex)	Specular microscopy	Decreased ECD Increased CV Decreased Ex
Lens	Lens opacity Linear value Peak 3D average	Pentacam – lens densitometry	All increase
Retina	Retinal nerve fibre layer (RNFL) thickness (average, superior, inferior, nasal, temporal quadrants)	Optical coherence tomography (OCT)	Thinner RNFL – all quadrants
	Retinal vessel calibre	Semi-automated retinal analysis software applied to fundus photographs	Reduced diameter of arterioles and arterio-venous ratio (AVR)
Systemic	Frailty status	Assessment of walking speed, grip strength, self-report of weight loss, exhaustion and low physical activity	Frailty status increases
	Non-frail (no criteria) Pre-frail (1–2 criteria) Frail: ≥3 of 5 criteria		

The unique access to and visibility of ocular tissues and range of visual functions permits investigation of a wide variety of physiological and pathological mechanisms. Many age-related ocular changes also have systemic associations or correlates of ageing in other end-organs or body systems but may be easier and less invasive to measure in the eye (Table 1). For example, changes in the lens, which has an extremely high protein content, may reflect systemic changes in protein structure and function in other organs (Truscott, 2010, 2011; Wormstone and Wride, 2011). Corneal endothelial cell parameters, lens density, retinal vessel calibre and thickness of the retinal nerve fibre layer (RNFL) are ocular parameters that vary with age that can be objectively and non-invasively imaged and assessed.

Ideally, proposed ocular biomarkers should be assessed in relation to established and validated BoA at a clinical or cellular level. Only two validated BoA, telomere length (TL) and CDKN2A expression, have so far been found to satisfy the majority of the criteria proposed by Baker and Sprott (1988). Telomeres are nucleoprotein complexes at the ends of eukaryotic chromosomes. Their DNA component shortens with somatic cell division and upon reaching a critically short length, a DNA damage signal leads to growth cycle arrest, resulting in replicative senescence (Saretzki and Von Zglinicki, 2002; von Zglinicki, 2002). Telomere shortening is associated with increasing chronological age and several pathologies, including cardiovascular disease (Starr et al., 2007) and renal dysfunction (Carrero et al., 2008). TL may be useful as a composite measure of healthy ageing, but not as a BoA when used in isolation (Der et al., 2012; von Zglinicki, 2012). Expression levels of the cell cycle regulator CDKN2A may represent a more robust BoA (Shiels, 2010). CDKN2A acts as a tumour suppressor and maintains cells in a state of growth arrest, both in replicative and stress induced-senescence. Increasing levels of CDKN2A transcriptional expression occur with increasing age and decreasing function of solid organs and peripheral blood leucocytes (PBLs) (Koppelstaetter et al., 2008; Krishnamurthy et al., 2004; Liu et al., 2009; McGlynn et al., 2009). However, there are limited data on how these parameters correlate with measures of physical frailty (Woo et al., 2008), a functional state characterised by an increased risk of multiple pathologies, low physical activity and slow motor performance (Fried et al., 2001). Frailty predicts cognitive and physical decline and is associated with an increased risk of morbidity and mortality, and may therefore act as a 'clinical' biomarker of ageing (Fried et al., 2001).

There are few data on biological ageing in sub-Saharan Africa, a region where the population of elderly people is rapidly expanding, and where the incidence of age-related non-communicable diseases is steadily increasing (Marquez and Farrington, 2012). The aim of this study was to investigate the association of a variety of ocular candidate BoA with 'systemic' BoA and frailty status in a South African adult population.

2. Methods

2.1. Study population

Individuals aged ≥30 years from an HIV prevention trials site in a township community of Cape Town, South Africa (Emavundleni Centre, Crossroads) were recruited as HIV-seronegative controls as part a case–control study investigating HIV and ageing (Pathai et al., 2012, 2013a). Socio-demographic information and medical history were obtained by interviewing participants in their first language (Xhosa or English). Data collected included factors known to affect ageing (e.g. UV exposure, smoking history). All participants underwent a full ophthalmic examination including measurement of visual acuity, evaluation by slit lamp microscopy and indirect ophthalmoscopy.

The study was approved by the Ethics Committees of the London School of Hygiene and Tropical Medicine and the University of Cape Town Faculty of Health Sciences, and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

2.2. Anthropometry, blood pressure and physical function including frailty assessment

Blood pressure (BP) was measured with a digital sphygmomanometer. Mean arterial blood pressure (MABP) was defined as two-thirds of the diastolic plus one-third of the systolic BP (Wong et al., 2003). Hypertension was defined as a systolic BP of 140 mmHg or higher, diastolic BP of 90 mmHg or higher, or the combination of self-reported high BP diagnosis and the use of anti-hypertensive medications (Wong et al., 2005). Body mass index (BMI) was defined as weight (in kilograms)/height^2.

Physical frailty was defined by the presence of ≥3 of 5 criteria: (i) unintentional weight loss (self reported and verified from clinic records where possible), (ii) self-reported low physical activity, (iii) self-reported exhaustion, (iv) weak grip strength and (v) slow walking time. Pre-frailty was defined as the presence of one or two of these criteria. Detailed information is available in the Supplementary Methods.

2.3. Blood-based biomarkers

2.3.1. DNA/RNA extraction

DNA was extracted from PBLs using the Maxwell™ Automated Purification System according to manufacturer's instructions (Promega, USA). DNA concentration and purity were quantified by Nanodrop Spectrophotometer (ThermoFisher Scientific, USA). RNA was extracted using Trizol reagent (Invitrogen, UK) following manufacturer's guidelines. DNA/RNA extraction was performed in Cape Town and samples shipped on dry ice to the University of Glasgow.