



# Anti-Aging Tests for Middle Aged Women

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The interest in aging and anti-aging research has increased significantly in recent years, leading to rapid expansion in the anti-aging market. Aging is associated with gradual physiological changes and an elevated risk of age-related ailments, and is divided into three categories: usual aging, successful aging, and pathological aging. Each category is associated with distinct implications for health and well-being. Middle-aged women who experience accelerated physiological changes that are intensified by hormonal changes during menopause are particularly vulnerable to chronic diseases. The importance of anti-aging tests is increasing since they enable early identification and intervention. Telomere length, oxidative stress markers, DNA repair markers, RNA profiles, inflammatory markers, hormone levels, and epigenetic changes are some molecular parameters studied to test for aging. In addition, a thorough review of middle-aged women's anti-aging profiles also includes monitoring the vitamin D levels and assessing the effects of endocrine-disrupting substances on ovarian aging. The application of personalized medicine paradigms, utilizing various diagnostic methods, will enable accurate risk prediction and the implementation of focused therapies, ultimately promoting the extension of health span and the improvement of quality of life in middle-aged women.

**Key Words:** Aging, Middle aged, Telomere

## INTRODUCTION

The general public and experts' great interest in aging increased several years ago because of increased global life expectancy, advances in science and technology, awareness of age-related diseases, and wellness and anti-aging trends. Studies on aging and industries are booming, and anti-aging businesses are growing explosively. According to the global anti-aging therapeutic market forecast for 2022, the average annual growth rate from 2023 to 2031 is predicted to be 17.5% [1].

Pathophysiologically, aging is a slow and irreversible process. Aging can be broadly divided into usual aging and successful aging. Usual aging refers to a typical change over time and does not include diseases or environmental effects. For example, changes such as loss of muscle mass, turbidity of lenses, reduction of ability to maintain balance, and decreasing thinking ability can

be mentioned. Alternatively, successful aging refers to a state in which physical and cognitive functions are normal (even not decreasing) and there are no age-related disabilities or diseases, as opposed to usual aging. Rowe and Kahn [2] added and defined active social activities such as participating in community events, engaging in volunteer work, or attending social gatherings, to it. For example, it refers to the aging of people with minimal physiological changes according to age [3,4]. Recently, as the importance of age-related diseases has been emphasized, pathological aging is also considered important. Pathological aging coexists with general aging and aging-related diseases [5]. In addition to an increased risk of several aging-related diseases, including neurological, cardiovascular, metabolic, musculoskeletal, and immune system disorders, there is a reduction in tissue and cell functions [6,7].

Received: April 10, 2024 Revised: September 6, 2024 Accepted: October 19, 2024

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## AGING IN MIDDLE-AGED WOMEN

### Why aging is important for middle-aged women

For both late middle-aged men and women, many bodily functions begin to decline rapidly. In particular, women are physically and physiologically more likely to develop chronic diseases due to rapid changes in the production and secretion of hormones in menopause and the acceleration of aging [8]. Previous studies [9–11] have showed a relationship between physical activity, muscular function, and aerobic power in older women, as well as circulating estrogen levels, insulin-like growth factor 1, and anabolic hormones such as dehydroepiandrosterone sulfate.

### Necessity of anti-aging tests for middle-aged women

In middle-aged women, age-related diseases, like cardiovascular disorder, osteoporosis, and dementia, could increase. Anti-aging tests can monitor and detect age-related disease early and intervention can be performed

[12,13].

The physical changes in women after menopause are all different. Aging can also show large differences between people. Therefore, personalized medicine predicts the risk of disease and determines the treatment for each individual. Anti-aging tests will be needed to diagnose and treat each disease quickly and accurately.

The maximum human life span is estimated to be 121 years [14]. However, in the past, people couldn't live until life expectancy due to various fatal diseases, but now the expectation of being able to live life expectancy is becoming a reality because of the development of medicine, nutrients, and other factors. Anti-aging tests can also contribute to extending life expectancy.

## ANTI-AGING TESTS FOR MIDDLE-AGED WOMEN

### Molecular biomarkers

Some molecular mechanisms that underlie aging sta-

**Table 1.** Aging tests: positive and negative aspects

| Anti-aging test                           | Positive aspect                                                                                                      | Negative aspect                                                                                                               |
|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| TL testing                                | Insights into cellular aging and potential health risks<br>TRF analysis is the gold standard                         | Expensive and technically challenging<br>Variability due to biological and methodological factors                             |
| DNA repair ( $\gamma$ -H2A.X analysis)    | Reliable marker for quantifying DNA damage<br>Established method for assessing aging-related DNA damage              | Requires specialized equipment and expertise<br>Complex interpretation                                                        |
| Epigenetic modification (DNA methylation) | Accurate age estimation through epigenetic clocks<br>Linked to age-related disorders like diabetes                   | High cost and complexity<br>Correlation with chronological age may vary                                                       |
| Transcriptome profiling (RNA-seq)         | High-resolution analysis of gene expression<br>Identifies age-related expression changes                             | Expensive and technically challenging<br>Requires advanced bioinformatics skills                                              |
| Non-coding RNAs (miRNAs)                  | Accessible biomarkers<br>Correlated with age-related changes and diseases                                            | Limited understanding of biological functions<br>Variability influenced by multiple factors                                   |
| Inflammation markers                      | Easy to measure and widely used<br>Reflects systemic inflammation                                                    | Non-specific; influenced by various factors<br>May not indicate underlying cause                                              |
| Hormone levels                            | Insights into hormonal imbalances and effects on aging<br>Melatonin useful for circadian rhythms                     | Fluctuations due to many factors<br>Requires specific timing                                                                  |
| EDCs                                      | Identifies exposure to harmful chemicals affecting reproductive health<br>Understands environmental impacts on aging | Complex testing; requires advanced techniques<br>Results may be difficult to interpret in the context of other health factors |
| Oxidative stress markers                  | Insights into oxidative damage and antioxidant status<br>Associated with various health conditions                   | Results vary with diet, lifestyle<br>Lack of standardization                                                                  |
| NLR                                       | Readily available and inexpensive<br>Reflects systemic inflammation and immune response                              | Influenced by a wide range of conditions<br>Less specific than other inflammation markers                                     |
| Vitamin D levels                          | Essential for bone health and well-being<br>Insights into age-related vitamin D production changes                   | Influenced by diet, sun exposure<br>Requires periodic monitoring                                                              |

TL: telomere length, TRF: terminal restriction fragment, RNA-seq: RNA sequencing, miRNAs: micro ribonucleic acids, EDCs: endocrine-disrupting chemicals, NLR: neutrophil-lymphocyte ratio.

tus may be reflected in molecular biomarkers (Table 1 and Fig. 1).

### DNA and chromosomes

**Telomere length testing:** The telomere repeat and sub-telomeric sequences that make up the terminal portions of eukaryotic chromosomes are among the genome's most changeable and quickly changing parts [15]. Telomeres are the protective caps at the end of chromosomes. The shortening of telomeres is associated with aging. Testing telomere length (TL) can provide insights into cellular aging and potential health risks. There are a lot of methods for TL testing: quantitative polymerase chain reaction, terminal restriction fragment (TRF) analysis, a variety of quantitative fluorescence in situ hybridization methods, single TL analysis, and telomere shortest length assay [14]. Among these, TRF is the 'gold standard' for TL measurements.

Many studies state that TL is related to female reproduction, particularly menopause [16]. In a previous paper, the average TRF length in the extended fertility group was significantly longer than in the normal fer-

tility group [16-19].

### DNA repair

$\gamma$ -H2A.X, CRAMP, EF-1a, stathmin, N-acetyl-glucosaminidase, and chitinase are serum makers of DNA damage [20]. Especially  $\gamma$ -H2A.X is a crucial component of DNA damage foci. Immunohistochemistry of  $\gamma$ -H2A.X is a well-established method for quantifying aging-related DNA damage [21,22].

### Epigenetic modification

Well-established aging indicators include age-related changes in DNA methylation patterns measured by the epigenetic clock. Three CpG sites that predict age with a mean deviation of less than five years were identified in a study that examined blood methylation profiles. Diabetes and other age-related disorders are linked to changes in DNA methylation [23].

### RNA and transcriptome

**Transcriptome profile:** Recent advancements in single-cell RNA sequencing (RNA-seq) technology have led

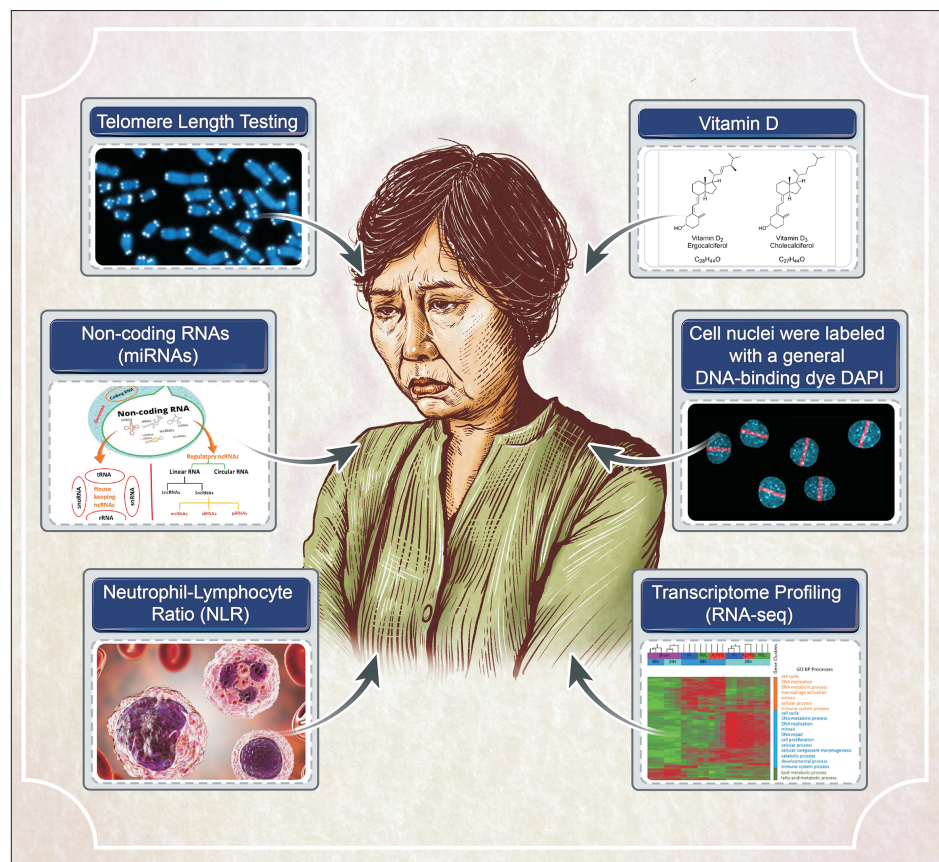


**Fig. 1.** Illustrated summary of anti-aging tests for middle-aged women. miRNAs: micro ribonucleic acids, DAPI: 4',6-diamidino-2-phenylindole dihydrochloride, RNA-seq: RNA sequencing.

to its application in studying aging biomarkers. Lu et al. [24] demonstrated that cell-to-cell expression variation, assessed through single-cell RNA-seq of high-dimensional flow cytometry sorted T cells, is linked to aging and susceptibility to diseases.

**Non-coding RNAs:** micro ribonucleic acids (miRNAs) are small non-coding RNAs that regulate diverse biological processes, including metabolism and aging, through base-pairing mechanisms. Circulating miRNAs, stable in plasma either by residing in exosomes or binding to proteins or lipoproteins, serve as accessible biomarkers. Age-related hearing loss in both humans and mice is correlated with miR-34a, the first circulating miRNA with changed expression throughout mouse aging [25]. Furthermore, miRNA-21 has been identified as an inflammatory biomarker in plasma, as evidenced by a study involving 365 miRNAs in healthy and elderly humans [26].

#### Inflammation markers

Tests like C-reactive protein, erythrocyte sedimentation rate, and cytokine levels assess inflammation in the body. Chronic inflammation is linked to various diseases, including cardiovascular issues and autoimmune conditions associated with age-related disease.

#### Hormone level

Hormone testing for middle-aged women focuses on various hormones that play key roles in their health and well-being. The hormones commonly tested and their significance are as follows: estrogen, progesterone, follicle-stimulating hormone, luteinizing hormone, thyroid hormones, cortisol, and testosterone. Hormone tests can be a part of anti-aging assessments as hormone imbalances can impact various aspects of aging.

Melatonin level is a gold-standard marker of the central circadian clock when measured from plasma or saliva and is defined as the time at which a salivary concentration of melatonin of 3 to 5 pg/mL is reached, which is expected to occur 2 to 3 hours before sleep onset [27]. Melatonin is much more efficient than vitamin C in reducing the extent of oxidative stress. Cardinali [28] suggested the supplementation of melatonin to counteract age-related declines in levels has shown promise in the prevention of neurodegenerative diseases. Melatonin offers various benefits, including its hypnotic, chronobiotic, and cytoprotective properties, which can aid in improving sleep latency, mitigating sundowning symptoms, stabilizing biological rhythms,

and minimizing inflammatory damage and neuronal loss to a limited extent [29].

#### Endocrine-disrupting chemicals and ovarian aging

Exposure to endocrine-disrupting chemicals (EDCs) has been associated with an increased risk of ovarian aging, resulting in diminished ovarian reserve, reduced fertility, irregular menstrual cycles, earlier onset of menopause, and premature ovarian insufficiency/failure in epidemiological studies [26,30]. Particularly involving EDCs like Bisphenol A, phthalates, parabens, and polychlorinated biphenyls, have confirmed the loss of ovarian follicles [31]. EDC testing is possible using mass spectrometry (MS) instruments such as liquid chromatography-MS/MS and inductively coupled plasma-MS. The mechanisms underlying these effects include interference with receptor-mediated pro-apoptotic pathways, induction of signal transduction and cell cycle arrest, and epigenetic modifications, ultimately impairing ovarian follicular development.

#### Oxidative stress markers

Tests that measure oxidative stress levels in the body can contribute to cellular damage and aging. Total antioxidant status (TAS)/total oxidant status (TOS) as the oxidative stress index (OSI) are laboratory tests used to measure oxidative stress in the body. Measures total antioxidant capacity based on the reduction of copper(II) to copper(I). Elevated levels of oxidants can indicate increased oxidative stress, which has been associated with various health conditions such as cardiovascular disease, cancer, and neurodegenerative disorders. The TAS is used to measure the overall antioxidant status of the body [32]. These assays are performed using different chemical reactions and techniques to quantify the levels of oxidants or antioxidants in biological samples such as blood or tissues. TOS and OSI are associated with the Reynolds Risk Score in the cohort of postmenopausal women [33]. Malondialdehyde, lipid peroxide, hydrogen peroxide, and reduced glutathione are other oxidative stress-related biomarkers.

#### Neutrophil-lymphocyte ratio in aging

The neutrophil-lymphocyte ratio (NLR) is a readily available measure of immunological response to various viral and non-infectious stimuli, as well as a systemic inflammatory indication [34]. Numerous factors, such as age, race, medicine, chronic illnesses like obesity, diabetes, heart disease, and stroke, as well as

diagnosis of mental illness, solid organ cancer, anemia, and stress, might affect NLR [35]. Patients with dry age-related macular degeneration had a slightly higher mean NLR than controls [36].

### Vitamin D and aging

The production and metabolism of vitamin D are influenced by aging, marked by factors such as decreased sun exposure and reduced skin capacity to produce vitamin D. The normal range of 25-hydroxy-vitamin D is between 20 and 40 ng/mL. Ultraviolet B, essential for the conversion of 7-dehydrocholesterol to previtamin D3 in the skin, decreases in concentration by over 50% from ages 20 to 80. Aged skin produces approximately 40% less vitamin D compared to younger skin [37,38]. Recent studies indicate an age-related reduction in vitamin D production of 13% per decade, resulting in a production at 70 years being half that at 20 years [39].

## CONCLUSION

In conclusion, interest in aging and anti-aging is constantly increasing. Anti-aging is an important issue in middle-aged women undergoing rapid hormonal changes and immune changes, and various tests are being performed.

## FUNDING

This work was supported by the Soonchunhyang University Research Fund.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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