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Specific alterations in the circulating levels of the SIRT1, TLR4, and IL7 proteins in patients with dementia

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ABSTRACT

Sirtuins have gained considerable attention as epigenetic regulators for slowing aging and age-related disorders. The growing association between neurodegeneration and inflammation has led researchers to investigate interactions of sirtuins with inflammatory markers in neurodegenerative diseases. We analyzed SIRT1's association with chronic inflammation in dementia as an age-related neurodegenerative condition through Toll-like receptor 4 (TLR4) and interleukin-7 (IL7) for the first time. In the present study, we observed a significant increase in the level of SIRT1 in patients with all types of dementia. Interestingly, the level of TLR4 protein was significantly lower in only the patients with Alzheimer's disease (AD) compared to the healthy elderly subjects. There was no significant change in the level of IL7 between the diseased and healthy elderly subjects. A significant positive correlation between SIRT1 level and age in healthy elderly subjects was evident according to Pearson's correlation test. However, this correlation was not observed in the dementia patients. Furthermore, the positive correlation between the levels of IL7 and TLR4 in the healthy elderly subjects was absent in the dementia patients. However, there was no direct association between the examined single nucleotide polymorphisms (SNPs) and dementia at the molecular level. According to logistic regression analysis, dementia risk increases 1.16 times due to an increase in the SIRT1 level and 24.23 times due to a decrease in the TLR4 level. Interestingly, a high level in the total antioxidant status (TAS) increases the risk of dementia approximately 33.32 times. Therefore, the current study, for the first time, provides a much better molecular understanding of the interaction between decreasing TLR4 levels and increasing SIRT1 levels in dementia, especially in AD. Furthermore, it highlights the importance of epigenetics in several age-related diseases and suggests that developing novel therapies to prevent or slow down the progression of dementia may support healthy aging.

1. Introduction

According to the World Alzheimer Report (2016), approximately 5% of the world's elderly population (46,8 million people) had dementia in 2015, and it is projected that this number will reach 131,5 million people in 2050. Alzheimer's disease (AD) is the most common form of dementia accounting for 60–70% of cases (WHO, 2015). According to the mortality statistics from the Turkish Statistics Institute, the proportion of elderly people who died from AD increased to 4% in 2014 from 2.7% in 2010. In addition, the prevalence rates of probable AD and dementia were calculated to be 11.0% and 20.0%, respectively, in the Turkish population (Gurvit et al., 2008). While AD has emerged

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as a major public health challenge for aging populations all over the world, the underlying molecular mechanisms of disease onset and progression have not yet been unraveled.

Irreversible neuronal loss in critical brain regions is a pathological hallmark of dementia, including AD (Tan et al., 2017). The accumulation of amyloid-beta (Aβ) plaques and the formation of neurofibrillary tangles containing hyperphosphorylated tau protein are thought to be possible causative mechanisms of these neuronal deaths (Hardy and Selkoe, 2002; Šimić et al., 2016). Until quite recently, the treatment strategy for AD pathogenesis has remained limited to the clearance of Aβ plaques to increase neuronal cell survival. However, the consequent lack of definitive therapy for disease progression has directed researchers to develop newer diagnostic and treatment strategies. As they are related to longevity and healthy aging, sirtuin proteins, especially SIRT1, and the other proteins in the sirtuin pathway are thought to be potential diagnostic and therapeutic molecules in dementia.

SIRT1, a NAD-dependent histone deacetylase, has a wide spectrum of metabolic and stress-tolerance properties due to its functions as a transcription factor and cofactor, in addition to being a target for histone and non-histone proteins (Tissenbaum and Guarente, 2001; Salminen et al., 2013; Shimoyama et al., 2011). SIRT1 protects cells against oxidative stress, regulates glucose/lipid metabolism, promotes DNA stability and slows down various age-related disorders, including neurodegenerative conditions, by binding to and deacetylating several substrates (Longo and Kennedy, 2006; Guarente and Franklin, 2011). Chromatin-, stress-, DNA repair-, and/or metabolism-related substrates, such as p53, NF-kB, FOXO, and PARP1, are examples of substrates from which SIRT1 transfers acetyl groups to an ADP-ribose molecule using NAD+ as an enzymatic co-factor (Martínez-Redondo and Vaquero, 2013). Furthermore, in neuronal cultures and animal studies, the neuroprotective role of SIRT1 and its therapeutic potential were previously reported in neurodegenerative diseases, including AD (Patel et al., 2005; Guarente, 2008; Bonda et al., 2011). Molecular studies showed that SIRT1 activation prevents the accumulation of Aβ plaques and tau pathology through the nuclear factor kappa B (NF-kB) signaling pathway by upregulation of the *ADAM10* gene, induction of the Notch pathway, and inhibition of the mTOR pathway (Chen et al., 2005; Song et al., 2011; Braidy et al., 2012). In addition, SIRT1 may regulate Aβ production by modulating BACE1 expression via NF-kB signaling (Kaltschmidt et al., 1999; Bourne et al., 2007; Buggia-Prevot et al., 2008). As shown in previous studies, SIRT1 epigenetically reprograms inflammation taking about AD formation at the earlier stages by altering transcription factors (Yeung et al., 2004; Xie et al., 2013; Hardy and Selkoe, 2002). In addition, it was observed that brains of AD patients have consistently reduced NAD+ levels and SIRT1 transcription and/or protein levels involved in chronic inflammation that can also be altered by increased levels of the activated proinflammatory transcription factor NF-kB (Qin et al., 2006; Serrano-Marco et al., 2012; Vachharajani et al., 2016). This mounting evidence points out the regulatory role of SIRT1 in inflammation during AD progression and/or prevention.

In one of our studies, we found a significant positive correlation between SIRT1 level and age, revealing enhanced SIRT1 expression with increasing age (Kilic et al., 2015). We thought that the high levels of SIRT1 protein might have a role in alleviating the oxidative stress that is significantly increased in the elderly. For the first time, we show in the present study the effects of SIRT1 on chronic inflammation in dementia through Toll-like receptor 4 (TLR4), which activates the NF-kB intracellular signaling pathway (Vaure and Liu, 2014) and interleukin-7 (IL7), an inflammatory cytokine. In addition, we investigated the association between *SIRT1* single nucleotide polymorphisms (rs7895833 A > G in the promoter region, rs7069102 C > G in intron 4 and rs2273773 C > T in exon 5 silent mutation) and the levels of SIRT1, TLR4, and IL7 expression, as well as the total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) with dementia in the Turkish population.

2. Experimental methods

2.1. Study groups

The study groups consisted of 80 randomly selected healthy elderly nursing home residents (age: 73.41 ± 10.02; range: 57–92) and 79 patients suffering from dementia (age: 73.19 ± 7.9; range: 58–94) including AD (n = 49) and other dementia-related conditions, such as progressive nonfluent aphasia (PFNA), progressive supranuclear palsy (PSP), among others (n = 30), who were recruited from the Neurology Department of Bezmialem Vakif University Hospital, Istanbul, Turkey. The parents of patients with a family history of dementia had no consanguinity.

The diagnosis of AD and other dementia-related conditions involved a two-step diagnostic process: screening for cognitive impairment with Folstein's Mini Mental State Examination scale (MMSE; scores #24) and confirmation by a detailed neurological examination, magnetic resonance imagining (MRI), assessment of daily living activities and neuro-psychological testing using the Clinical Dementia Rating Scale and the Blessed Dementia Rating Scale. AD was also diagnosed as per NINCDS-ADRDA criteria. However, the severity of AD was not indicated in the present study.

This study was approved by the Ethical Committee of Bezmialem Vakif University (08.04.2013/No: 36-13). All of the participants, after giving their written informed consent, completed a structured questionnaire in order to collect demographic data. The study was conducted in accordance with the ethical principles described by the Declaration of Helsinki.

2.2. Determination of the SIRT1, TLR4, and IL7 protein levels

After 12 h of fasting, blood samples from subjects were taken into tubes (Vacuette, Greiner Labor technic, Germany) and centrifuged for 5 min at 4 °C. Then, the blood serum and plasma were collected and stored at −20 °C. The plasma/serum samples of the subjects were analyzed for the levels of circulating SIRT1, TLR4, and IL7 proteins using enzyme-linked immunosorbent assay (ELISA) kits from USCN Life Science. Briefly, standards and samples were incubated in antibody-coated 96-well plates for 2 h. After incubation, the substrate solution was added, and the plates were incubated for 15–25 min. After addition of stop solution, the intensity of the color change in each well was measured in a microplate reader at 450 nm (Chromate Manager 4300, Palm City/USA).

2.3. Measurement of the total antioxidant and oxidant status and determination of the oxidative stress index

The total antioxidant status (TAS) and the total oxidant status (TOS) of the serum were determined using an automated measurement method by an automated analyzer (Chromate Manager 4300, Palm City/USA) as described earlier (Erel, 2004, 2005). In the measurement of TAS, the absorbance of colored dianisidyl radicals was monitored to determine the rates of a Fenton reaction, which initiates free-radical reactions resulting in the production of a hydroxyl radical. Then, the antioxidative effect of the sample against potent free-radical reactions was measured in terms of mmol equiv/L Trolox (Rel Assay Diagnostics, Gaziantep/TURKEY).

In the measurement of TOS, the total amount of oxidant molecules in the sample was determined by measuring the intensity of the colored complex produced by the reaction of ferric ions, which were oxidized in a ferrous iono-dianisidine complex due to the presence of oxidants, with xylenol orange in an acidic medium. The calibration of the assay was performed with hydrogen peroxide, and the TOS values are expressed in terms of micromolar hydrogen peroxide (H_2O_2) equivalents per liter (μmol H_2O_2 equiv/L) (Rel Assay Diagnostics, Gaziantep/TURKEY).

The oxidative stress index (OSI) was determined using the formula