

Decreased HDAC1 Gene Expression in Patients with Alzheimer's Disease

Ali Bayram¹, Feridun Akkafa², Ahmet Ozer² and Remzi Yigiter³

¹*Firat University, Elazig High School of Health Sciences, Elazig, Turkey*

²*Harran University Faculty of Medicine, Department of Medical Biology, Urfa, Turkey*

³*Gaziantep University Faculty of Medicine, Department of Neurology, Gaziantep, Turkey*

E-mail: ¹<ak.ali@hotmail.com>, ²<feridunakkafa@yandex.com>, ²<axozer@gmail.com>
³<yigiter@gantep.edu.tr>

KEYWORDS Alzheimer's Disease. HDAC1. mRNA Expression. RT-PCR

ABSTRACT One of the commonest neurodegenerative diseases, Alzheimer's disease (AD), is characterized by progressive decline in memory and cognitive functions with inexorable neurodegeneration in brain. Histone-tail acetylation have been known to be associated with some crucial neurologic functions, thus, the enzymes regulating this events such as HDAC1 are associated with such neurodegenerative diseases like AD. The research objective was to document the levels of HDAC1 expression in peripheral lymphocytes harvested from patients clinically diagnosed with AD. Fifty patients diagnosed with AD, and 49 age- and sex-matched controls were recruited to the study. Total RNA was extracted, cDNA was synthesized, and HDAC1 expressions were tested using quantitative real-time polymerase chain reaction (qRT-PCR). HDAC1 expression was found significantly attenuated in patients with AD compared with the controls ($p < 0.001$). The research data suggested that lower expression levels of HDAC1 may have an impact in the etiology or disease course of AD.

INTRODUCTION

Histone acetylation is an important regulatory mechanism of chromatin remodeling by taking role in various biological mechanisms through fundamental alterations in gene expression (Roth et al. 2001). In those acetylation-deacetylation events, histone deacetylases (HDACs), together with histone acetyl transferases (HATs), act as a rheostat to maintain homeostasis in protein acetylation and transcription events with pivotal roles especially in neurons (Langley et al. 2005; Kazantsev and Thompson 2008). HDACs often function as gene expression suppressors via dictating heterochromatin formation, which is a tightly packed, condense version of chromatin with certain Lysine-Arginine residues remained deacetylated on core histone proteins silencing gene expression (Gallinari et al. 2007). Our current knowledge of the effect of histone acetylation on neuronal activities was mostly achieved by the studies on HDACs and their inhibitors (HDACi). Histone tail acetylations have been known to be associated with some crucial neurological functions

including memory formation, neuronal plasticity and learning (Alarcón et al. 2004; Korzus et al. 2004; Vecsey et al. 2007). Human HDAC proteins are divided into four main classes, of which class-I and II HDACs are mostly studied and implicated with central nervous system and related neurodegenerative disorders (Alarcón et al. 2004). HDAC1 belongs to the class I HDACs, and it is majorly implicated with memory function of fear extinction.

HDAC inhibitors improve the memory formation in murine models of Alzheimer's disease (AD) suggesting that histone acetylation is associated with memory (Alarcón et al. 2004; Fischer et al. 2007; Govindarajan et al. 2011). It was suggested by proteome based HDACi studies that HDAC1 and HDAC2 can be more potent HDACs in memory formation. Those two closely work together, and have nearly identical structures carrying crucial developmental roles in neuronal differentiation (Montgomery et al. 2009; Salisbury and Cravatt 2007). DNA damage and neuronal death are among the underlining etiopathogenesis factors of many neurodegenerative disorders including AD, and studies indicated that they can be prevented by increased HDAC1 activity (Kim et al. 2008).

AD is characterized by progressive decline in memory and cognitive functions with inexorable neurodegeneration in brain eventually causing dementia. The majority of dementia cases

Address for correspondence:
Dr. Ali Bayram
Firat University Elazig High
School of Health Sciences,
Elazig, Turkey
E-mail: ak.ali@hotmail.com

are diagnosed with AD (Qiu et al. 2009). It is one of the leading causes of mortality and morbidity in elderly. It was estimated that there were over 35 million individuals with dementia worldwide in 2010 and social impact and economic burden of this alignment is increasing (Wortmann 2012). AD and dementia represent a significant health care problem for Turkish society as well. A cross-sectional, population based study reported that AD prevalence in a Turkish metropolis reached 11% in population over 70 years old, which is comparable to that of the statistics with the Western European counterparts (Gurvit et al. 2008). Underlying neuropathological alterations include extracellular amyloid plaques and intracellular tangles causing progressive dysfunction and death of nerve cells in brain. The actual causative factor of the disease is unknown, but many possible risk factors are spelled including inflammation, oxidative stress, genetics and epigenetics (Mattson 2004). In the etiology, variable combinations of genetics and environmental factors are plausible especially for the late-onset AD covering over 90% of the patients (Zawia et al. 2009).

Against growing body of studies aiming to understand the nature of the disease and develop therapeutic measures, AD is still a crucial social and health issue of our aging community. Epigenetic changes are accounted for one of the important way of molecular regulatory mechanisms, and may contribute to the AD pathogenesis [as reviewed in (Fisher A 2014)]. Thus, epigenetics studies promise a new scope in AD and dementia research for the development of better diagnostic and therapeutic solutions. In the present study, the researchers aimed to determine HDAC1 expression levels at mRNA level in patients with AD and compare the results with those in healthy volunteers.

METHODOLOGY

Disease Diagnosis and Samples Collection

In the present study, the researchers recruited fifty elderly patients (age >58 years) with the

diagnosis of AD who applied to the Department of Neurology at Gaziantep University School of Medicine. The total of forty-nine healthy individuals without any neurodegenerative disease from the same ethnic origin and geographical area with the patients were included to the study as the control group. Informed consent was obtained from all participants (informed consent was obtained from the family in case of incompetent patients with AD). The study is approved by the institutional ethics committee at Gaziantep University.

Quantitative Real-Time PCR (qRT-PCR)

RNA was obtained from blood samples of patients and controls. cDNA was made from RNA samples using reverse transcriptase PCR technique. Isolation of RNA from the blood samples was performed using *Pure Link RNA Mini Kit* (Ambion-Life Technologies, USA, Catalog Number: 12183-018A). cDNA synthesis from RNA samples was performed using *High-Capacity cDNA Reverse Transcription Kit* (Applied Biosystems, California, USA). In determination of the expression of target genes, the primer sequences and probes used are shown at Table 1. Primer sequences were designed according to the recommendations at the NCBI website.

The researchers used an online, web-based tool for designing of the primers and probes (www.roche-applied-science.com/sis/rtpcr/upl), and validated the accuracies of the obtained sequences with the NCBI nucleotide sequences software. Primers were synthesized by IDT (Integrated DNA Technologies, Belgium). The researchers used probes of Universal Probe Library (UPL) in the study (Roche Diagnostics, Mannheim, Germany). UPL probed in short sequences of nucleotides with 8-9 bp, the locked nucleotides (Locked Nucleic Acid; LNA) technology was used. 20 ul of qPCR reaction was prepared [14 ml of distilled water, 0.2 ml of probe

Table 1: Housekeeping gene *Beta Actin* and the sequences and probes of target genes

| Gene | Primer sequence | NCBI accession number |
|-------|---|-----------------------|
| ACTB | 5'- ACCACACCTTCTACAATGAGC-3 5'- CAGCCTGGATAGCAACGTAC-3 | NM_001101.3 |
| HDAC1 | 5'- TCTGGCTTCTGTTACGTC AATG -3 5'- ATATCCCGTAGGTCCCCAG -3 | NM_004964.2 |

UPL (10 mM), 0.4 ml of forward primer (10 mM), 0.4 ml of reverse primer (10 mM), 4 ml of the Lightcycler TaqMan master (Roche Diagnostics, Mannheim, Germany), 1 ml of the sample mixture of cDNA]. The prepared mixture was transferred to the capillary tubes and placed in the LightCycler®2.0 real-time PCR instrument (Roche Diagnostics, Mannheim, Germany). PCR reaction was setup according to the following protocol: 10 minute denaturation at 95° C, 10s denaturation at 95° C for 45 cycles of amplification, 30s binding at 60° C, 60s elongation at 72° C. At the last stage, a loop kept at 40° C for 30s, and the PCR reaction was terminated.

HDAC 1 expression levels were normalized to the corresponding β -actin gene expression levels, and calculated relative to the mean level in those acquired from the healthy controls by the comparative CT method (Livak and Schmittgen 2001).

Statistical Analysis

Data is expressed as bars exhibiting the means \pm standard error of means (SEM). Statistical analysis was performed using Minitab 17.1

(Minitab, Inc., Coventry, UK). Comparison of the measures was performed with the 2-sample Student's t-test.

RESULTS

Patient group consisted of 50 patients diagnosed with AD, and their age range was between 43 and 89 years. Control group was formed by 49 sex- and age-matched, healthy volunteers, and their age range was between 61 and 81 years. The demographic characteristics of the study participants were depicted in Table 2.

Table 2: Demographics of the study participants

| | AD | Control |
|------------------------|------------------|------------------|
| Number of Patients (n) | 50 | 49 |
| Age (year) \pm (SD) | 74.32 \pm 8.94 | 72.33 \pm 5.56 |
| Sex (Female/Male) | 29/21 | 25/24 |

Here, the researchers evaluated HDAC-1 expression levels in AD patients and controls to test its potential effect in disease course. AD patients have significantly lower HDAC1 expression levels than those in the control subjects (Fig. 1; $p < 0.001$)

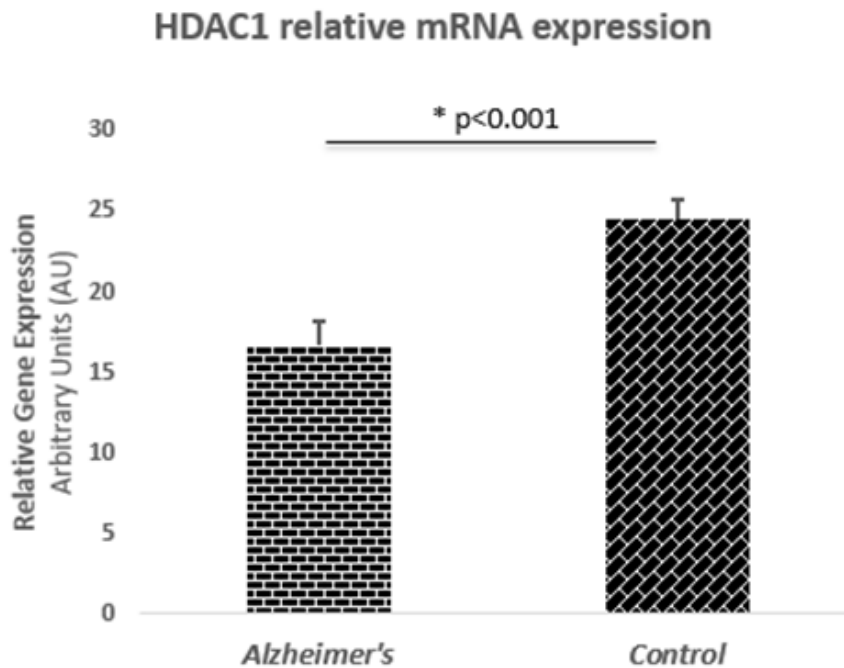


Fig. 1. Relative histone deacetylase-1 mRNA levels in AD patients and control individuals

DISCUSSION

Alzheimer's disease is a chronic disorder characterized with a progressive and irrepressible neurodegeneration along with associated clinic features including deterioration in memory and cognitive functions. It is the most common dementia-related neurodegenerative disorder affecting patients with advanced age. Recent studies have shown that epigenetic regulation, especially histone tail acetylation, is associated with the pathogenesis of AD (Lee and Ryu 2010; Szyf M 2014). Deletion studies have indicated that individual HDACs of class I (widely expressed in mammalian tissues) can affect the expression of limited sets of genes ranging from tumor suppressor genes to transcription factors (Witt et al. 2009). In a clinical study, (Al-sadany et al. 2013), it was reported that HDACs activity levels were significantly increased in patients with AD in moderate and severe condition compared to the age- and sex-matched healthy controls (Al-sadany et al. 2013). They explored that altered HDACs level was correlated with patients' cognitive functions. Data suggested that HDACs may have negative impact on disease course via silencing the expression of critical genes organizing neuronal activities such as cognition, memory and neuronal growth (Strahl and Allis 2000). The outcome of the present study suggested that patients with AD have decreased levels of HDAC1 expressions comparing with the healthy, age-matched controls. This conflicting data may be due to their measurement of total HDAC activity instead of the individual activity via characterization of the each HDAC. The important point to remember for HDAC activities is that various specific functions of HDACs are dependent of the substrates they affect in specific tissues (Fischer et al. 2010). HDAC1 belongs to class-1 HDACs and ubiquitously expressed in tissues. The researchers did not check the activity of HDAC1 in peripheral lymphocytes as well. It is suggested that it may be a worthy target to conduct activity studies on HDAC1 in AD patients.

In another study (Guan et al. 2009), focusing on memory formation, the researchers have shown that the elevated expression of HDAC2, one of the class-I HDACs, negatively affected neuronal plasticity and memory formation events in a mice model of AD. In the same study, they found that neuronal synapse number and mem-

ory consolidation increased in HDAC2 knock-out mice. In mice with attenuated expression of HDAC2 via chronic HDACi treatment, synapse number and learning were moderately restored. HDAC1 overexpression was not found to be related with the memory formation and learning in that study. Those results suggested that HDAC2 is more implicated with memory-related pathways in AD mice model, and against their close structural and working relationship, gene regulatory pathways of HDAC1 and HDAC2 can differ distinctly (Guan et al. 2009; Hassig et al. 1998). It has been documented that current therapeutic approaches with HDAC inhibitors may just slow the progression and provide a moderate enhancement in cognitive parameters and memory formation, (Francis et al. 2009; Kilgore et al. 2010; Ricobaraza et al. 2012). In the present study, attenuated expression level of one of the class-1 HDACs, HDAC1, was detected in the patients with AD suggesting that not just overexpression, but also decreased expression of HDAC1 may have a clinical importance yet to develop better diagnostic as well as therapeutic approaches.

It's known that DNA damage and related neuronal cell death are among the underlining factors in some neurodegenerative diseases including AD (Adamec, Vonsattel, and Nixon 1999; Kruman et al. 2004). In a transgenic mice study (Kim et al. 2008), it has been stated that increased cell cycle activity, DNA damage and eventual neurotoxicity arose with HDAC1 loss of function through p25-CK cascade. Low HDAC1 levels and neuronal cell death were attributed as possible pathological events in neurodegeneration suggesting the critical cellular functions of HDAC1 in neurons (Kim et al. 2008). Class-1 HDACs have explicit roles in neural system including transcriptional repression of several genes, the inhibitory function of especially HDACs 1 and 2 to assure the expression of neural proteins in neuronal tissues as landscaping regulators (Huang et al. 1999). Attenuated expression of HDAC1 in patients with AD correlates with this transgenic mice study suggesting that low HDAC1 levels may negatively affect neuronal survival, one of the hallmark findings of AD. It is also worthy to mention here that HDAC inhibitors possessing anti-HDAC1 activity (and other class-1 HDACs) generally have strong apoptosis-inducing and proliferation inhibitory effects in cells suggesting the importance of those HDACs in cell survival (Witt et al. 2009).

CONCLUSION

The present study found that patients with AD have lower mRNA expression levels of HDAC1 comparing with their healthy counterparts. More clinical studies individually determining HDAC activities in patients with AD are required to figure out new alterations and their clinical importance in the etiology of AD.

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