# Serum Doublecortin-like Kinase-1 Levels in Age-Related Macular Degeneration

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## ABSTRACT

**Purpose:** To assess the serum levels of doublecortin-like kinase-1 (DCLK-1) in dry and wet-type age-related macular degeneration (AMD) patients and compare with those of control subjects.

**Material and Methods:** This cross-sectional case control study was conducted on a total of 62 subjects consisting of 13 wet-type (exudative) and 19 dry-type (atrophic) AMD patients and 30 control subjects. Serum DCLK-1 level was assessed by enzyme-linked immunosorbent assay (ELISA) kit in all participants. Independent samples-t test and one-way ANOVA were used to compare mean serum DCLK-1 levels between groups.

**Results:** Mean age for control and AMD groups were 68.9±4.8 and 70.8±5.1 years respectively (p:0.14). Male/Female ratio was 15:15 in control group and 17:15 in AMD group. Mean serum DCLK-1 level was 1.24±0,71ng/mL in control, and 0.79±0,52 ng/mL in AMD groups (p:0.006). Mean serum DCLK-1 level for dry and wet-type subgroups were 0,76±0,52 and 0,83±0,54 ng/mL respectively (p:0.70). There was no statistically significant difference between wet-type AMD and control groups (p: 0.134). In the dry-type AMD subgroup mean serum DCLK-1 level was significantly lower than the control group (p:0.031).

**Conclusion:** Serum DCLK-1 level was significantly lower in AMD group compared to control group. Further studies including genetic and immunohistochemical analyses are needed to investigate the potential pathophysiological role of DCLK-1 in AMD.


**Key words:** Doublecortin-like kinase-1, Age-related macular degeneration, Sserum doublecortin-like kinase-1 level.

## INTRODUCTION

Age-related macular degeneration (AMD) is currently considered the leading cause of visual disability among elderly population in western countries. Its pathogenesis, likely multifactorial, involving a complex interaction of metabolic, functional, genetic and environmental factors, remains poorly understood.[1] There are two classical forms of AMD: the "dry" or atrophic and the "wet" or exudative forms. The dry form is characterized by progressive dysfunction of the retinal pigment epithelium (RPE), photoreceptor loss, and retinal degeneration. The wet form is is characterized by choroidal neovascularization with intraretinal or subretinal leakage, hemorrhage, and RPE detachments.[2] Clinical hallmark of AMD is the degeneration of RPE cells, a process that associates with the accumulation of oxidative stress (OS)-derived lysosomal lipofuscin, impairing lysosomal degradation, and the presence of extracellular protein/lipid deposits (drusen) between the basal lamina of the RPE and the inner collagenous layer of the Bruch's membrane.[3] The capacity to prevent the accumulation of cytotoxic protein aggregates and the ability to remove them by autophagy decline in aged cells, particularly in postmitotic RPE cells.[4] Autophagy, literally, 'self eating', is an intracellular degradation process that clears long-lived proteins, aggregated proteins, lipid droplets and organelles from the cytoplasm in eukaryotic cells.[4,5] Emerging studies suggest the regulation of autophagy by several protein kinases with particular emphasis on serine/threonine protein kinases such as mTOR, AMP-activated protein kinase, mitogen-activated protein kinase (MAPK).[6]

Doublecortin like kinase-1 (DCLK-1) is a serine/threonine-

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**Received:** 12.10.2019
**Accepted:** 14.10.2019
*Ret-Vit 2020; 29: 101-104*
DOİ:10.37845/ret.vit.2020.29.18

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protein kinase encoded by the DCLK1 gene located on 13q13.3. The protein contains two N-terminal doublecortin domains, which bind microtubules (MT) and regulate MT polymerization, a C-terminal serine/threonine protein kinase domain, which shows substantial homology to Ca2+/calmodulin-dependent protein kinase, and a serine/proline-rich domain in between the doublecortin and the protein kinase domains, which mediates multiple protein-protein interactions.[7] DCLK-1 is also a microtubul-associated protein (MAP) involved in dynamics and organization of MTs during several cellular processes, including neuronal migration, retrograde transport, neuronal apoptosis and neurogenesis.[7,8,9] DCLK-1 was first identified in the brain.[10] Recently, evidence has emerged indicating that DCLK-1 regulates biological processes outside the central nervous system. It has been shown to be highly expressed in the stromal and epithelial compartments in colon and pancreatic cancer as well as Barrett's esophagus and esophageal adenocarcinoma.[11] Associated with optic nerve vertical cup-to-disc ratio, DCLK-1 is also reported to be a possible pathogenic gene for primary open-angle glaucoma, normal-tension glaucoma and high-tension glaucoma[12] Since protein kinases are integral for the autophagy clearance system DCLK-1 might also have a possible role in AMD pathogenesis. We therefore compared the serum DCLK-1 levels of wet and dry-type AMD patients with those of control subjects.

## MATERIALS AND METHODS

This cross-sectional case control study was conducted on a total of 62 subjects consisting of 13 wet-type (exudative) AMD and 19 dry-type (atrophic) AMD patients and 30 controls. The control group included randomly selected age and sex matched volunteers who had no AMD disorder. A detailed medical history was obtained and full ophthalmic examination including fluorescein angiography and optical coherence tomography was performed in all patients. The International ARM Study Group Classification was used to diagnose and classify AMD.[13] Exclusion criteria for both AMD patients and control subjects were known malignancy, ocular, local or systemic infection or inflammation, any systemic disease other than hypertension, intravitreal drug injection within the last 3 months, and the use of any topical or systemic medication that could interfere with serum DCLK-1 measurement. Ethics approval was obtained from the Ataturk University Institiutional Review Board. After informed consent, 5 cc of peripheral venous blood sample was obtained from each participant and the serum was was separated from the cells by centrifuging at 3000g for 10 min. The samples were then kept at -80°C until the time of study.

### Enzyme-linked immunosorbent assay (ELISA)

The serum DCLK-1 level was quantified using a commercially available ELISA assay (USCN Life Science Inc., Wuhan, China). The 96-well plate coated with monoclonal antibody against DCLK-1 was pre-blocked. Purified DCLK-1 protein at different concentrations (0–10 ng/ml) was used to create a standard curve. Serum samples were diluted 1:4 and 1:10 with PBS. The diluted serum samples along with the purified DCLK-1 proteins were added into the pre-blocked 96-well plate and incubated for two hours at room temperature. The plate was then incubated with biotinylated polyclonal antibody against DCLK-1 for one hour at room temperature. After three washes, the plate was then incubated with Streptavidin conjugated with horseradish peroxidase (HRP) for thirty minutes at room temperature. Finally, the plate was developed with HRP substrate for twenty minutes and terminated by adding stop solution. The value of OD 450 nm was measured using a microplate reader and the concentration of DCLK-1 in serum samples was determined based on the standard curve constructed using purified DCLK-1.

### Statistical Analyses

Statistical analysis was performed using SPSS for Windows version 21.0 (SPSS, Chicago, IL). Independent samples-t test and one-way ANOVA were used to compare mean serum DCLK-1 levels between groups. Fisher exact test was used to compare ratios as appropriate. Statistical significance was accepted at a p value of $<0.05$.

## RESULTS

There was no significant difference among the groups in terms of mean age and gender distribution ($p>0.05$). Mean age for control and AMD groups were 68.9±4.8 and 70.8±5.1 years respectively (p:0.14). Male/Female ratio was 15:15 in control group and 17:15 in AMD group. Serum DCLK-1 level was comparable between male (n:17) and female (n:15) subgroups (p:0.195) in AMD patients. Mean serum DCLK-1 level was 1.24±0,71ng/mL in control and 0.79 ±0,52 ng/mL in AMD groups (p:0.006). Mean serum DCLK-1 level for dry and wet subgroups were 0,76±0,52 ng/mL and 0,83±0,54 ng/mL respectively (p:0.70). There was no statistically significant difference between wet-type AMD and control groups (p: 0.134). In the dry-type AMD subgroup mean serum DCLK-1 level was significantly lower than the control group (p:0.031).

## DISCUSSION

Autophagic processes in post-mitotic cells such as neurons, cardiac myocytes, and RPE, are essential for cellular quality control, as these cells are unable to decrease

their load of damaged organelles, accumulated lipids or protein aggregates through cell division.[14-16] During aging, autophagy is critical to remove damaged components that would otherwise accumulate and catalyze the formation of cytotoxic molecules *in situ* under oxidative stress.[16,17] Dysregulated autophagy contributes to accumulation of intracellular debris, inflammasome activation and cell death.[16,18-20] Numerous age-related-degenerative disorders are associated with autophagic dysregulation and decreased autophagy including Alzheimer's disease(AD), Parkinson's disease and AMD.[16,21-23] The Alzheimer's-associated Amyloid beta (Aβ) group of misfolding proteins were reported to accumulate in ageing retinas.[24] These deposits were found in retinal whole-mounts associated with photoreceptor outer segments, the RPE and Bruch's membrane.[25,26] Serum DCLK-1 level was reported to be significantly higher in AD compared to controls.[27] Since there is a similarity in terms of histopathological basis with AD, serum DCLK-1 levels might also change in AMD.

The RPE supports photoreceptor cell renewal by daily phagocytosis of shed photoreceptor outer segments (OS). The daily ingestion of these lipid-rich OS imposes a constant degradative burden on these terminally differentiated cells.[16] These cells rely on Microtubule-Associated Protein 1 Light Chain 3 (MAP1LC3) family of proteins for phagocytic clearance of the ingested material.[16] DCLK-1 is another MAP which is a member of the microtubule-associated doublecortin (DCX) family. Dcx-microtubule effects have been extensively studied, including recent studies showing cooperative binding effects and changes in microtubule structure, as well as regulation of molecular motors.[28-30] DCLK-1 might be involved in MT dynamics and organization among RPE cells.

In here, we aimed to evaluate serum DCLK-1 levels of patients with AMD in comparison with healty subjects. We found that serum DCLK-1 level was significantly lower in those who have the disease. Among those with AMD, serum DCLK-1 levels were comparable between wet and dry-type subgroups. DCLK-1 might have diagnostic, therapeutic, and prognostic value in AMD. Further evaluation including genetic and immunohistochemical analyses with larger subject numbers is needed to understand the potential pathophysiological role of DCLK-1 in AMD.

**Conflict of interest:** The authors declare that they have no conflict of interest.

**Funding:** None

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