European Review for Medical and Pharmacological Sciences 2016; 20: 4196-4201

Correlation between serum melatonin and aMT6S level for age-related macular degeneration patients

X.-D. LV[1,2], S. LIU[3], Z. CAO[4], L.-L. GONG[2], X.-P. FENG[2], Q.-F. GAO[2], J. WANG[5], L. HU[6], X.-C. CHENG[2], C.-H. YU[2], Y.-Q. XING[1]

[1]Eye Center, Renmin Hospital of Wuhan University, Wuhan, Hubei Province, China
[2]Department of Ophthalmology, Xianning Central Hospital, the First Affiliated Hospital of Hubei University of Science and Technology, Xianning, China
[3]Department of Pediatrics, Xianning Central Hospital, the First Affiliated Hospital of Hubei University of Science and Technology, Xianning, China
[4]Department of Radiology, Xianning Central Hospital, the First Affiliated Hospital of Hubei University of Science and Technology, Xianning, China
[5]Department of Ophthalmology, People's Hospital, Sanshui district, Foshan City, Guangdong Province, China
[6]Department of Science-Education, Xianning Central Hospital, the First Affiliated Hospital of Hubei University of Science and Technology, Xianning, China

Abstract. – OBJECTIVE: To analyze the levels of serum melatonin (MLT) and assay of 6-sulfatoxymelatonin (aMT6S) of age-related macular degeneration (AMD) patients and study their correlation with AMD risk factors.

PATIENTS AND METHODS: 58 AMD cases were selected and 58 healthy cases of the same time period were selected according to 1:1 closest matching method. ELISA method was used to test serum MLT and aMT6S level.

RESULTS: Levels of MLT and aMT6S in AMD group were lower than those in the control group, and differences were statistically significant ($p < 0.05$). Based on analysis of AMD subgroup, differences on gender had no statistical significance compared with AMD type. For cases with smoking, cardiovascular disease and corrected visual acuity lower than 0.1, MLT and aMT6S levels were reduced at 0.05). Through the regression analysis, we concluded that smoking history, cardiovascular disease history, best corrected visual acuity, MLT and aMT6S level were independent risk factors, among which MLT [OR = 3.624 (odds ratio: OR)] and aMT6S (OR = 3.201).

CONCLUSIONS: MLT and aMT6S may be related to the incidence of AMD.

Key Words:
Age-related macular degeneration (AMD), Melatonin, aMT6S, Smoking, Best corrected visual acuity, Correlation.

Introduction

Age-related macular degeneration (AMD) is a kind of chronic degenerative change at the macular area of eye ground, causing central vision loss[1]. It is a kind of aged disease and the second cause of blindness in western people over 50 years old right after diabetic ocular fundus pathology[2]; its prevalence rate is 2% and accounts for 50% in all blind cases[3]. With accelerated aging in China, positive rate and prevalence rate of AMD are increasing by year[4]. Its causes are not clear yet, but long term chronic photopathy, age, hereditary, cardiovascular disease, smoking and malnutrition may be main causes[5]. Cell injury caused by ROSoxidative stress) may be an important pathogenesis[6]. Melatonin (MLT) is an indoleamine hormone that has multiple physiological functions such as adjusting biological rhythms, regulating blood pressure, antioxidant, anti-apoptosis, cell protection, immunoregulation and anti-tumor[7-9]. In recent years, a significant function of MLT in terms of maintaining retinal function was gradually recognized. Retina is one of its main natural secreting sections in the human body. MLT is also able to control pigment of eyes and regulate the amount of light that reaches photoreceptor[10]; it was able to clean hydroxyl radical and protect retinal pigment epithelium (RPE) cells

Corresponding Author: Yi-Qiao Xing, MD; e-mail: xingyiqiao530530@sina.com

from oxidative damage[11]. Therefore, it is predicted that physiological loss of MLT for old people is the important cause for PRE functional damage, which causes AMD at last. At present, studies abroad have shown that serum MLT and urine assay of 6-sulfatoxymelatonin (aMT6S) levels for AMD patients are lower than those of healthy people[12]. Consequently, an ergogenic supplement of MLT or similar matter is good for AMD treatment[13]. Nevertheless, there is still no reports regarding the correlation between MLT and AMD in China. With that as a basis, this study aimed at analyzing serum MLT and urine aMT6S level in patients suffering AMD and studying their correlation with AMD risk factors, which will provide a theoretical foundation for MLT as the etiological treatment of AMD.

Patients and Methods

Patients

58 AMD cases diagnosed in our hospital from Jan 2015 to Jan 2016 were selected in accordance with AMD diagnostic criteria made by Ocular Fundus Disease Group of Chinese Medical Association in the 2nd National Ocular Fundus Disease Academic Conference in 1986. *Exclusion criteria:* (1) Secondary macular degeneration, eye traumas, eye surgery history and other clear eye diseases; (2) Factors influencing MLT level, such as recent smoking, drinking, oral administration of non-steroid anti-inflammatory drug, antipsychotic drug, antianxiety drug, sleeping pills, tryptophan or □ receptor blocking pharmakon within 12h and MLT intake, etc.; (3) The collected specimen is not qualified.

The current study has been approved by patients and their family members through Ethics Committee of our hospital. 1:1 closest matching method for age and gender was used to select 58 healthy cases of the same time period. For AMD group, 30 male and 28 female cases with ages ranging 52 to 75 years old (average value of 63.4 ± 12.7 years old) were selected; the course of disease was from 1 month to 2 years and average period was 10.5 months; 23 cases with best corrected visual acuity lower than 0.1 and 35 cases more than 0.1; there were 19 smoking cases and 26 cases with cardiovascular disease history. They were divided into atrophic type (that is "dry") and exudative type (that is "wet") based on clinical and pathological manifestations. Atrophic type refers to atrophic lesions at macular region caused by atrophy of choriocapillaris, thickening of the glassy membrane and epithelial atrophy of retinal pigment, and its clinical symptoms were pigment disorder at macular region, drusen and RPE atrophy. Exudative type refers to RPE atrophy, in which the glassy membrane was damaged and choroidal blood vessels grow under the retina and form new tunica vasculosa. Fundus manifestations were edema, exudation, diastasis of retinal neuroepithelium layer or RPE layer, macular hemorrhage and organized hyperplasia, etc. Among them, there were 27 dry AMD cases and 31 wet cases. In the control group, there were 29 male cases and 29 female cases with ages between 50 and 76 years old and the average value is (63.2 ± 13.3) years old. 19 cases with naked vision lower than 0.3 and 39 cases more than 0.3 but the best corrected visual acuity for all is higher than 0.5; there are 20 smoking cases and 23 cases with cardiovascular disease history. Differences are of no statistical significance between two groups in terms of smoking and cardiovascular disease history cases.

Testing Method

ELISA method was used to test plasma MLT and urine aMT6S levels and all kits are purchased from R&D Company (Minneapolis, MN, USA). All procedures were made strictly in accordance with instructions. MLT collection time was at night (00:00). Collect about 5 ml peripheral venous blood into the anticoagulative tube and centrifuge it for 15 min at 3000 r/min. Take the supernatant and keep it in -70°C refrigerator for testing. Take about 10 ml morning (08:00) urine with an empty belly and kept in -70°C refrigerator for further testing.

Statistical Analysis

The SPSS19.0 statistical software (SPSS Inc., Chicago, IL, USA) is used for data processing. The measurement data was represented by mean value ± standard deviation. The comparison between groups was made through the *t*-test. The enumeration data is presented as case or percentage. The comparison between groups was made through the $\square^2$-test. The logistic testing model was used in multi-factor regression analysis. $p < 0.05$ was considered as statistically significant.

Results

Comparison Between MLT and aMT6S Level

Average MLT and aMT6S level in AMD group were lower than those in the control group and differences are of statistical significance ($p < 0.05$) (Table I).

Analysis on MLT and aMT6S Level in AMD Subgroup

Based on analysis of AMD subgroup, differences were statistically no significant in terms of comparison between gender and AMD type. For cases with smoking, cardiovascular diseases and corrected visual acuity lower than 0.1, their MLT and aMT6S level were significantly decreased ($p < 0.05$) (Figure 1).

Multi-factor Logistic Regression Analysis

Taken all together, gender, age, smoking history, cardiovascular disease history, best corrected visual acuity, MLT and aMT6S as independent variables and AMD as an inclusive model of the dependent variable, we concluded that smoking history, cardiovascular disease history, best corrected visual acuity, MLT and aMT6S were independent risk factors (Table II).

Table I. Comparison between MLT and aMT6S levels.

Group	MLT (pg/ml)	aMT6S (ng/ml)
AMD group	89.7 ± 23.2	5.5 ± 2.7
Control group	162.4 ± 24.9	9.3 ± 3.4
t	6.534	6.428
p	< 0.001	< 0.001

Discussion

It is proved in studies[12,13] that MLT level in blood and urine was decreasing for aged people and lowered MLT level *in vivo* circulation was related to some geriatric diseases such as cancer, coronary artery disease and Alzheimer's disease. As for the correlation between MLT and AMD,

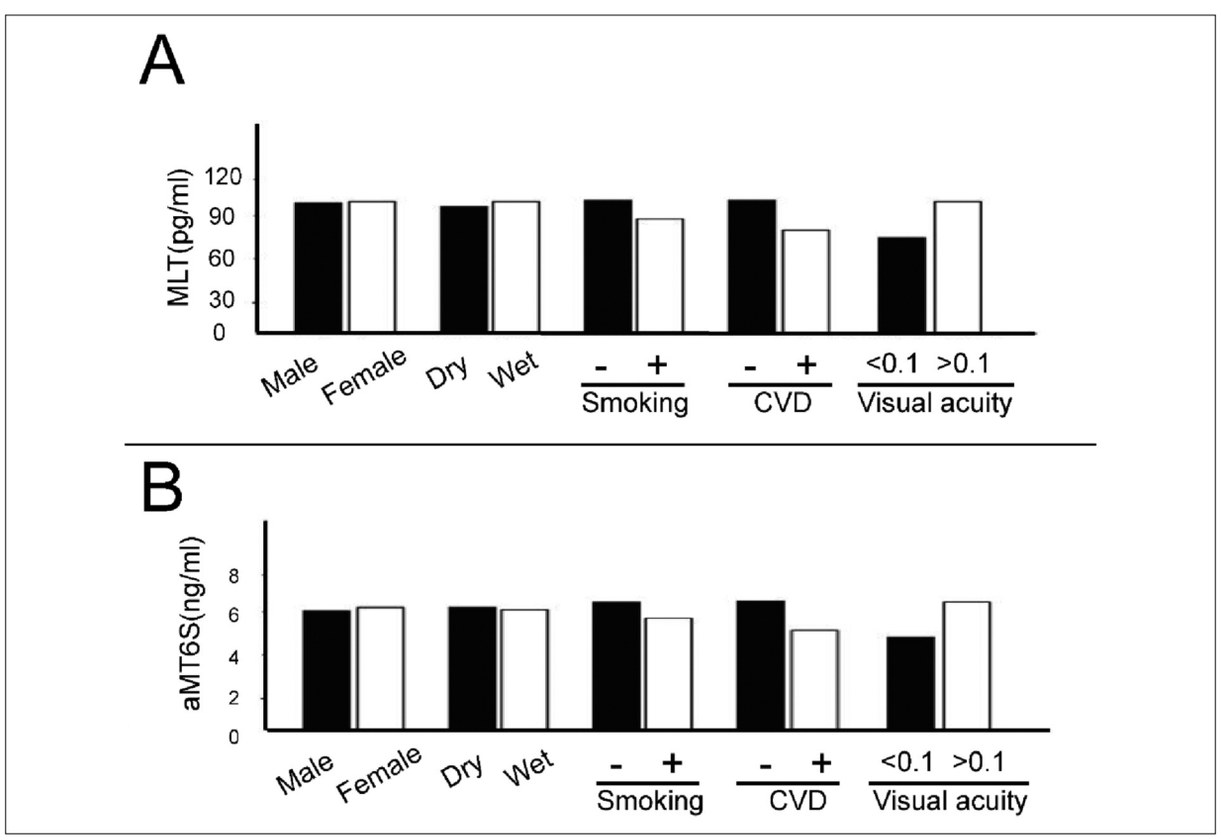

Figure 1. MLT and aMT6S levels in AMD subgroup. ***A,*** MLT; ***B,*** aMT6S. CVD: cardiovascular disease.

Table II. Multi-factor logistic regression analysis.

Factors	β	Wald	p	OR	95% CI
Smoking history	0.102	3.627	0.041	1.234	0.634-2.698
Cardiovascular disease history	0.234	3.468	0.034	1.326	0.328-3.623
Best corrected visual acuity	0.217	3.129	0.008	1.765	0.129-2.964
MLT	0.357	5.646	< 0.001	3.624	3.127-5.639
aMT6S	0.421	5.532	< 0.001	3.201	2.867-5.724

Liang et al[14] found out through intervening with different concentration of MLT in oxidative stress model of epithelial cells of retinochrome induced by hydrogen peroxide that MLT was able to increase activity of antioxidant enzyme (SOD, GR and GSH-px) and, therefore, relieved oxidative damage of RPE cells. In oxidative stress model of RPE cell, there were changes in mitochondrion DNA. MLT intervention was able to relieve oxidative damage of mitochondrion DNA and protect RPE cells[15]. In addition, MLT was able to reduce damages to rod outer segment of photoreceptor from free radical caused by sunlight[16]. After an investigation, Rosen et al[17] found out that as the main metabolite of MLT, aMT6S levels of AMD patients were able to reflect serum MLT levels, which were (6.24 ± 3.45) ng/ml and it was 40% lower than (10.40 ± 4.51) ng/ml in the control group. Yi et al[13] made an investigation and found that after oral medication of 3 mg MLT every day for three months, pathological changes of maculopathy for 70% AMD patients (including both dry and wet) were relieved. So, it could be concluded that MLT was able to protect the retina and delay macular degeneration. It was proven through Rastmanesh's studies[18] that MLT was able to treat or prevent AMD.

Recently, the function of oxidative stress in AMD course of disease was getting more attention. When the body is through all kinds of noxious stimulus, the content of highly reactive molecules in the human body such as ROS and RNS increased and the degree of oxidation was more than the removal of oxide, which causes an imbalance of oxidative system and antioxidant system. As a result, a large amount of reactive oxygen was generated and it was beyond removal capacity of the antioxidant system. The human body will form oxidative stress status and cause DNA oxidative damage and abnormal protein expression. Finally, it will cause cytotoxic effect and irreversible damage to the body[19]. Cell apoptosis plays an important role in many histopathological processes such as body growth and development and aging. Causes for apoptosis include aging, virus infection and oxidative damage[20]. Some authors[21] found that oxidative stress caused by reactive oxygen was an important link in cell apoptosis. Rod outer segment of retinal photoreceptor contains lots of polyunsaturated fatty acids and it was easily attacked by oxidative damage, which further enable cytolysis chain to begin[22]. In the early stage of AMD, pressure on RPE cells was increasing with age; the degradation of photoreceptor enzyme pieces were generated during RPE phagocytosing lysosome and degradation products include retinal induced complex, which was featured by photoinduction, increases oxidative stress damage, interferes RPE cell metabolism, caused obstacle for oxygen diffusion and induced new vessels[23]. MLT was a strong free-radical scavenger, which was able to remove highly toxic cyano group and other oxygen atom group and improve antioxidant enzyme level[24]. It mainly depended on 5 methoxy on indole ring and synergistic effect from acetyl on side chain in order to prevent MLT from degradation by monoamine oxidase. It was demonstrated that physiological content of MLT was negatively correlated to age[25]. MLT content was reducing over 45 years old and also it was a high incidence period for AMD. Therefore, physiological MLT is an important factor for RPE dysfunction, which may be the main cause for AMD[26].

Conclusions

We observed that MLT and aMT6S level in the AMD group were lower than those in the control group, which was consistent with previous studies. Based on AMD subgroup, the differences had no statistical significance regarding gender and AMD type. MLT and aMT6S levels were lower for cases of smoking, cardiovascular diseases and corrected visual acuity less than 0.1, which indicated that MLT might be closely relat-

ed to high-risk factors for AMD. Through the regression analysis, we concluded that smoking history, cardiovascular disease history, best corrected visual acuity, MLT and aMT6S level were independent risk factors, among which OR values of MLT and aMT6S level are high. Age and gender matching method was used in the study to exclude the age, which was an important influencing factor. Above all, MLT and aMT6S may be related to the incidence of AMD.

Acknowledgements

Grant support was provided by the Youth Talent Project of Science and Technology Research Program of Hubei Provincial Department of Health of China. Grant Number: QJX2012-50.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Dag E, Ornek N, Ornek K, Gunay F, Turkel Y. Mini mental state exam versus Montreal cognitive assessment in patients with age-related macular degeneration. Eur Rev Med Pharmacol Sci 2014; 18: 3025-3028.
2) Ong BB, Ah-Fat FG. Age-related macular degeneration. Br J Hosp Med (Lond) 2016; 77: C18-21.
3) Kim JH, Chang YS, Kim JW, Lee DW, Han JI, Kim CG. Self-recognition of recurrences among patients with exudative age-related macular degeneration. Clin Exp Optometry 2016; 99: 56-60.
4) Ye Z, Shuai P, Zhai Y, Li F, Jiang L, Lu F, Wen F, Huang L, Zhang D, Liu X, Lin Y, Luo H, Zhang H, Zhu X, Wu Z, Yang Z, Gong B, Shi Y. Associations of 6p21.3 region with age-related macular degeneration and polypoidal choroidal vasculopathy. Sci Rep 2016; 6: 20914.
5) Yoo P, Carlone D, Ren LY, Lam WC. Assessment of online health resources for ophthalmology patients with age-related macular degeneration or diabetic retinopathy. Can J Ophthalmol 2016; 51: e1-2.
6) Fanjul-Moles ML, López-Riquelme GO. Relationship between oxidative stress, circadian rhythms, and AMD. Oxid Med Cell Longev 2016; 2016: 7420637.
7) Demirtas CY, Pasaoglu OT, Bircan FS, Kantar S, Turkozkan N. The investigation of melatonin effect on liver antioxidant and oxidant levels in fructose-mediated metabolic syndrome model. Eur Rev Med Pharmacol Sci 2015; 19: 1915-1921.
8) Liu LF, Qin Q, Qian ZH, Shi M, Deng QC, Zhu WP, Zhang H, Tao XM, Liu Y. Protective effects of melatonin on ischemia-reperfusion induced myocardial damage and hemodynamic recovery in rats. Eur Rev Med Pharmacol Sci 2014; 18: 3681-3686.
9) Aranda ML, González Fleitas MF, De Laurentiis A, Keller Sarmiento MI, Chianelli M, Sande PH, Dorfman D, Rosenstein RE. Neuroprotective effect of melatonin in experimental optic neuritis in rats. J Pineal Res 2016; 16: 14-15.
10) Stefanova NA, Zhdankina AA, Fursova AZh, Kolosova NG. Potential of melatonin for prevention of age-related macular degeneration: experimental study. Adv Gerontol 2013; 26: 122-129.
11) Zetner D, Andersen LP, Rosenberg J. Melatonin as protection against radiation injury: a systematic review. Drug Res (Stuttg) 2016; 20: 56-57.
12) Tosini G, Baba K, Hwang CK, Iuvone PM. Melatonin: an underappreciated player in retinal physiology and pathophysiology. Exp Eye Res 2012; 103: 82-89.
13) Yi C, Pan X, Yan H, Guo M, Pierpaoli W. Effects of melatonin in age-related macular degeneration. Ann N Y Acad Sci 2005; 1057: 384-392.
14) Liang FQ, Green L, Wang C, Alssadi R, Godley BF. Melatonin protects human retinal pigment epithelial (RPE) cells against oxidative stress. Exp Eye Res 2004; 78: 1069-1075.
15) Rosen RB, Hu DN, Chen M, McCormick SA, Walsh J, Roberts JE. Effects of melatonin and its receptor antagonist on retinal pigment epithelial cells against hydrogen peroxide damage. Mol Vis 2012; 18: 1640-1648.
16) Lundmark PO, Pandi-Perumal SR, Srinivasan V, Cardinali DP. Role of melatonin in the eye and ocular dysfunctions. Vis Neurosci 2006; 23: 853-862.
17) Rosen R, Hu DN, Perez V, Tai K, Yu GP, Chen M, Tone P, McCormick SA, Walsh J. Urinary 6-sulfatoxymelatonin level in age-related macular degeneration patients. Mol Vis 2009; 15: 1673-1679.
18) Rastmanesh R. Potential of melatonin to treat or prevent age-related macular degeneration through stimulation of telomerase activity. Med Hypotheses 2011; 76: 79-85.
19) Nita M, Grzybowski A. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid Med Cell Longev 2016; 2016: 3164734.
20) Chen M, Rajapakse D, Fraczek M, Luo C, Forrester JV, Xu H. Retinal pigment epithelial cell multinucleation in the aging eye--a mechanism to repair damage and maintain homoeostasis. Aging Cell 2016; 15: 23-24.
21) Kim S, Kim YJ, Kim NR, Chin HS. Effects of Bevacizumab on Bcl-2 expression and apoptosis in retinal pigment epithelial cells under oxidative stress. Korean J Ophthalmol 2015; 29: 424-432.
22) Wang Y, Hanus JW, Abu-Asab MS, Shen D, Ogilvy A, Ou J, Chu XK, Shi G, Li W, Wang S, Chan CC.

NLRP3 Upregulation in retinal pigment epithelium in age-related macular degeneration. Int J Mol Sci 2016; 17. pii: E73

23) Marazita MC, Dugour A, Marquioni-Ramella MD, Figueroa JM, Suburo AM. Oxidative stress-induced premature senescence dysregulates VEGF and CFH expression in retinal pigment epithelial cells: Implications for Age-related Macular Degeneration. Redox Biol 2016; 7: 78-87.

24) Emamgholipour S, Hossein-Nezhad A, Ansari M. Can melatonin act as an antioxidant in hydrogen peroxide-induced oxidative stress model in human peripheral blood mononuclear cells? Biochem Res Int 2016; 2016: 5857940.

25) Rosen R, Hu DN, Perez V, Tai K, Yu GP, Chen M, Tone P, McCormick SA, Walsh J. Urinary 6-sulfatoxymelatonin level in age-related macular degenerationpatients. Mol Vis 2009; 15: 1673-1679.

26) Schmid-Kubista KE, Glittenberg CG, Cezanne M, Holzmann K, Neumaier-Ammerer B, Binder S. Daytime levels of melatonin in patients with age-related macular degeneration. Acta Ophthalmol 2009; 87: 89-93.