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Research Article

AGE-REALTED MACULAR DEGENERATION AND SERUM LEPTIN: A CASE–CONTROL STUDY

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ABSTRACT

Objective: The objective of this study was to evaluate the association of serum leptin levels with presence and severity of age-related macular degeneration (AMD).

Methods: A hospital-based case–control study conducted on 84 patients (42 cases and 42 controls) of either sex complaining of diminution of vision aged 40 years and above between August 2019 and 2021.

Results: Mean serum leptin level in cases was 1.55±0.99 ng/mL and control was 2.11±0.88 ng/mL ($p<0.008$). The serum leptin level was negatively correlated with early (1.06±0.99 ng/mL), intermediate (0.50±0.08 ng/mL), and dry age-related macular degeneration (ARMD) (0.29±0.12 ng/mL). However, we found higher serum leptin levels in exudative ARMD (2.58±0.99 ng/mL). Maximum number of cases were of exudative AMD (42.86%) followed by early AMD (33.34%) and intermediate and dry AMD (11.91% each).

Conclusion: We found a decrease in serum leptin levels as disease severity increases from the early AMD to intermediate AMD and Dry AMD. However, the levels of serum leptin levels were higher in patients with exudative AMD. This may be due to some pathological process that is different in dry and exudative AMD. This can form the basis for further research in future.

Keywords: Age-related macular degeneration, Choroidal neovascular membrane, Geographic atrophy, Serum leptin level.

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INTRODUCTION

Age-related macular degeneration (AMD) is a leading cause of irreversible loss of vision in the world. It is common in people more than 50 years of age [1]. AMD has been classified into two main types DRY AMD (non-exudative and non-neovascular), being geographical atrophy as the advanced stage of it; and WET AMD (exudative and neovascular) characterized by choroidal neovascularization, retinal pigment epithelium detachment, polypoidal choroidal vasculopathy, and retinal angiomatous proliferation. When neovascularization occurs, there is accumulation of hemorrhage, fluid, and lipid exudation within the macula which can culminate in the form of fibrosis called as DISCIFORM SCAR [2].

According to age-related eye disease study (AREDS) classification, AMD has been categorized as No AMD (AREDS category 1) is characterized by no or few small drusen (<63 μm in diameter). Early AMD (AREDS category 2) is characterized by a combination of multiple small drusen, few intermediate drusen (63–124 μm in diameter), or mild RPE abnormalities. Intermediate AMD (AREDS category 3) is characterized by any of the following features: Numerous intermediate drusen, at least one large drusen (≥125 μm in diameter), and geographic atrophy (a sharply demarcated, usually round or oval, area of atrophy of the RPE not involving the center of the fovea). Advanced AMD (AREDS category 4) is characterized by one or more of the following (in the absence of other causes) in one eye: geographic atrophy of the RPE involving the foveal center and neovascular maculopathy [3].

Leptin, a product of ogbene on chromosome 7q31.3, a 16-kDa produced by mRNA directed protein synthesis [4]. It is a protein secreted predominantly by the adipose tissue, has been proposed to play a significant role in healthy aging and protection against several neuronal disorders. Acting through hypothalamic receptors, leptin regulates food intake and energy balance [5-7]. Extracellular deposition of amyloid-β (aβ), the hallmark of Alzheimer disease (AD), has also been observed in the drusen of AMD patients. Leptin had been shown to promote β-amyloid clearance and chronic leptin treatment has been shown to improve memory function in mice models of aging and AD [8]. Elevated levels of serum leptin had been shown to be negatively associated with dementia and AD in several studies [9-11]. This negative correlation of serum leptin had also been found with AMD in the previous studies [12-15].

METHODS

It was a hospital-based prospective case–control study comprised 84 patients (42 cases and 42 controls) aged 40 years and above of either sex. Permission from the Ethical Committee and informed written consent of study population was taken. One eye of each patient with higher stage of ARMD was taken for the study.

The duration of study was 2 years. Immunocompromised and with other ocular diseases having CNVM were also excluded from the study. After taking history from patient complaining of diminution of vision, each patient was subjected to complete ophthalmic examination, clinical examination, and laboratory investigations including visual acuity testing, fundus examination with direct ophthalmoscopy, slit lamp biomicroscopy using 90D and indirect ophthalmoscopy, fundus photography, optical coherence tomography, macular function test with amsler grid, and routine laboratory investigations (fasting blood sugar, renal function test, liver function test, and lipid profile). After confirmation of diagnosis, grading of disease according to AREDS classification and after obtaining informed consent 2 mL of venous blood was drawn in non-fasting state in plain vial and was send to Multi-Disciplinary Research Unit of the hospital. Whole blood was placed at room temperature for 2 h and centrifuged at 4000 rpm for 20 min. Serum was separated from the blood sample. This extracted serum was stored at −80°C. The collection and storage process was same for cases and controls. Serum leptin levels were measured by human leptin ELISA kit based on sandwich technique. Serum leptin concentration was measured from optical density through standard curve.

Collected data were entered in the Microsoft Excel spreadsheet, coded appropriately, and later cleaned for any possible errors. The statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp. Armonk, NY, USA). All data collected and analyzed by qualitative Chi-square and quantitative t-test. All tests were performed at a 5% level of significance; thus, an association was significant if $p<0.05$.

RESULTS

A total of 45 patients were enrolled in the study of either sex aged 40 years and above in each group. Three samples of cases out of these got hemolyzed so excluded from the study. Thus, an overall of 42 cases and 42 controls were taken in this study.

Maximum 42.86% cases were in 61–70 year age, whereas minimum 4.76% were in 81–90 year age group. Fifty percentages of controls were in 40–60 year age group, whereas minimum 9.52% were in 81–90 year age group. Maximum 57.14% cases whereas 54.67% controls were males. About 76.19% cases and 73.81% controls had normal body mass index (BMI) (Mean BMI – cases 21.89±3.51 and controls 22.41±3.03 [p>0.05]). Statistically significant difference (p<0.021) was found only in hypertensive patients (Table 1).

Mean serum leptin levels were 1.55±0.99 ng/mL in cases, whereas, 2.11±0.88 ng/mL in controls, the difference between two groups was statistically significant (p<0.008) (Fig. 1).

Serum leptin levels at different age in two groups were statistically insignificant. In males, the serum leptin levels between two groups were also found to be statistically insignificant (p<0.236) butin females mean serum leptin level between cases and controls was found to be statistically significant (p<0.006). In cases maximum, mean serum leptin level 1.70±0.99 ng/mL was found in patients with normal BMI, whereas minimum 0.36±0.34 ng/mL in underweight group. In controls, maximum serum leptin levels 2.56±0.88 ng/mL was found in overweight BMI group, while minimum 1.91±0.76 ng/mL in obese group. This difference between serum leptin levels at different BMI level in two groups was statistically insignificant except for overweight group (p<0.021). The difference between serum leptin levels in different systemic illness in two groups was statistically insignificant (Table 2).

Maximum serum leptin level 2.58±0.99 ng/mL (1.81–2.90 ng/mL) was found in exudative type, followed by 1.06±0.99 ng/mL (0.51–2.31 ng/mL) in the early ARMD and 0.50±0.08 ng/mL (0.41–0.60 ng/mL) in intermediate ARMD, whereas minimum 0.29±0.12 ng/mL (0.09–0.40 ng/mL) in dry ARMD. The difference between serum leptin levels in different types of ARMD was statistically significant (p<0.0001) (Table 3).

Maximum prevalence of ARMD 42.85% was seen in 61–70 years age group, whereas minimum 4.76% in 81–90 year age group. Maximum early ARMD 57.14% was in 40–60 year age, whereas minimum 7.14% in 71–80 year age group. Maximum intermediate ARMD 40% was seen in 61–70 and 71–80 year whereas minimum 20% in 40–60. Maximum exudative 55.67% was in 61–70 year whereas 60% dry. Maximum number of cases is of exudative AMD (42.86%) followed by early AMD (33.34%) and 11.91% in intermediate and dry AMD (Fig. 2).

DISCUSSION

Age-related macular degeneration is defined as a common, chronic, and progressive degenerative disorder of the macula resulting from abnormalities in the retinal pigment epithelium, photoreceptors, and Choroidal-Bruch's membrane complex. Leptin is a protein thought to negatively affect the progression of AMD in previous studies.

In INDEYE study, aging was found as the most consistent non-modifiable environmental risk factors for AMD [12]. According to Jennifer R. Evans, age and genetic make-up of individuals are the most important risk factors for ARMD [13]. In our study also, maximum 42.86% cases were in 61–70 year age, whereas minimum 4.76% were in 81–90 year age group. The prevalence of AMD increased from 40 to 70 years and then decreased afterward. This finding was also consistent with INDEYE study. This can be attributed toward the un ability of older patients to attend eye examination and less life expectancy in developing countries.

Table 1: Sociodemographic profile

Age (years)	Cases (n=42)		Control (n=42)	
	No.	%	No.	%
40–60	14	33.33	21	50.00
61–70	18	42.86	11	26.19
71–80	8	19.05	6	14.29
81–90	2	4.76	4	9.52
Sex				
Male	24	57.14	23	54.76
Female	18	42.86	19	45.24
BMI (kg/m^2)				
<18.5 (underweight)	2	4.76	1	2.38
18.5–24.9 (normal)	32	76.19	31	73.81
25.0–29.9 (overweight)	5	11.90	7	16.67
≥30 (obese)	3	7.14	3	7.14
Systemic illness				
HTN	15	35.71	5	11.90
DM	11	26.19	6	14.29
BA	6	14.29	5	11.90

BMI: Body mass index

Table 2: Association of serum leptin level with different parameters

Age (years)	Cases (n=42)		Control (n=42)		p-value
	Mean	SD	Mean	SD	
40–60	1.40	0.98	2.04	0.86	0.052
61–70	1.69	0.99	2.34	0.75	0.076
71–80	1.61	1.00	1.79	0.80	0.735
81–90	0.58	0.09	2.32	0.83	0.051
Sex					
Male	1.53	0.99	1.86	0.88	0.236
Female	1.58	0.99	2.41	0.70	0.006*
BMI (kg/m^2)					
<18.5 (underweight)	0.36	0.34	2.54	1.09	0.115
18.5–24.9 (normal)	1.70	0.99	2.01	0.88	0.192
25.0–29.9 (overweight)	1.26	0.92	2.56	0.73	0.021*
≥30 (obese)	1.53	1.04	1.91	0.76	0.474
Systemic illness					
HTN	1.54	0.99	2.23	0.91	0.185
DM	1.63	0.99	1.84	0.73	0.658
BA	1.64	0.99	1.80	0.76	0.776

BMI: Body mass index

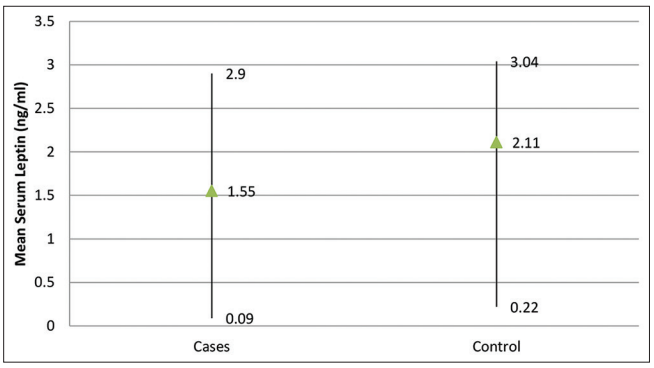

Fig. 1: Mean serum leptin levels

Table 3: Association of serum leptin level with stages of ARMD

Mean serum leptin level (ng/mL)			
ARMD	**Mean**	**SD**	**Range**
Early AMD	1.06	0.98	0.51–2.31
Intermediate AMD	0.50	0.08	0.41–0.60
Exudative AMD	2.58	0.99	1.81–2.90
Dry AMD	0.29	0.12	0.09–0.40

ARMD: Age-related macular degeneration

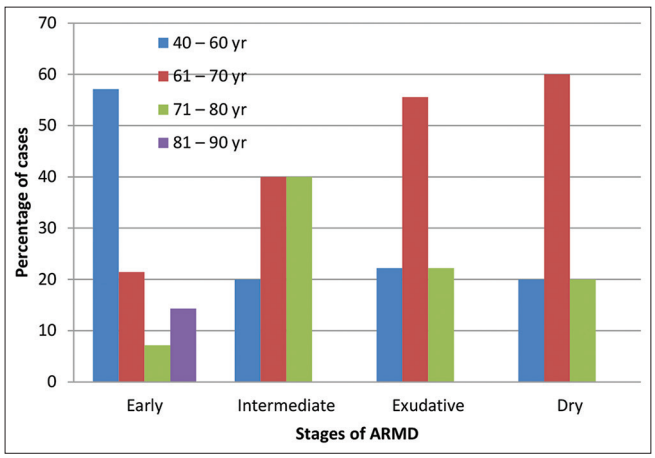

Fig. 2: Association of ARMD with age

Everekliogl u found that age and sex ratios were not substantially different among patients of ARMD and control subjects [14]. In INDEYE study also, no sex predisposition was found. Similarly, in our study, maximum 57.14% cases, whereas 54.67% controls were male and both groups were comparable in terms of gender [15].

In study by Everekliogl u [16], mean BMI was observed between patients with maculopathy and control subjects were 22.35±0.66 and 22.61±0.77, respectively (p>0.05). Qian-Y-Jhang *et al.* observed effect of overweight and obesity as risk factors for age-related macular degeneration and found that excess body weight was weakly associated with increase in the risk of AMD [17]. In our study, we found no association of BMI with AMD and difference was statistically insignificant (>0.05).

Seshasai *et al.* [2] observed that there was no statistically significant difference between cases and controls, in terms of the sex, diabetes and hypertension status, smoking, and BMI. Similarly, in our study also, we found no statistical significant association with diabetes and bronchial asthma. However, hypertension was found to be significantly associated (p<0.021).

In our study, mean serum leptin levels were 1.55±0.99 ng/mL in cases, whereas 2.11±0.88 ng/mL in controls. We were unable to find normal serum leptin levels in healthy adults in literature from best of our knowledge.

Everekliogl u [14] found that across all patients with maculopathy, serum leptin concentrations were significantly (p<0.001) lower (6.01±2.55 ng/mL) when compared with those in control subjects (13.21±2.27 ng/mL). Seshasai *et al.* [2], at Singapore Epidemiology of Eye Diseases Study, found that participants with AMD had lower serum leptin levels as compared to those without AMD. Similarly, in our study, the difference between two groups was statistically significant (p<0.008). However, serum leptin levels were less in general as compared to above mentioned studies in our population both in cases and controls. This can be due to difference in demographic profile, socioeconomic status, and ethnicity of patients.

According to study conducted by Isidori *et al.* [16], in adults of different BMI, serum leptin gradually declines with age. This decline is higher in women compared to men, but is also independent from BMI and other age-related endocrine changes. In our study also, the difference between serum leptin levels at different age in two groups was statistically insignificant. Although clinically, we found decline in serum leptin levels from 61 year onward in cases. This decline was not found in controls.

Seshasai *et al.* [2] found serum leptin levels to be significantly higher in women compared to men, a finding consistent with the previous literature that also report a 2 to 3 times higher concentration of serum leptin in females compared to males. Sex-specific difference in serum leptin levels could be explained by differences in body fat composition and due to the effect of sex hormones. In our study, no significant interactions were observed in the association between serum leptin and AMD by sex. However, when we compared it between cases and controls in either sex, the difference of mean serum leptin level in males was statistically insignificant (p<0.236), while this came out to be significant in females (p<0.006).

Saunier *et al.* [17], in their cohort, suggests a high risk for incident early AMD in individuals with high plasma high-density lipoprotein cholesterol levels. However, in our study, we found no correlation between lipoprotein cholesterol levels and serum leptin.

Hirvelä *et al.* [18] found a higher risk of ARM with the increase in BMI, whereas Klein *et al.* [19] demonstrated no associations between BMI and ARMD. Everekliogl u [14] found that having a BMI either lower or higher than the accepted normal range was associated with a significantly increased risk of maculopathy. In addition, comparable increases in risk were apparent for late-ARMD. Seshasai *et al.* [2] found no significant interactions between serum leptin and AMD by BMI. Similarly, in our study also, the difference between serum leptin levels at different BMI level in two groups was statistically insignificant except for overweight group (p<0.021). This can be due to regulatory effect of serum leptin level on lipid metabolism which could be more in overweight group, while, in obese group, this difference was not significant. We found serum leptin levels more in controls than cases, but this difference was not statistically significant.

Seshasai *et al.* [2] found insignificant association of serum leptin levels with systemic illness. In our study, we also found that the difference between serum leptin levels in different systemic illness in two groups was statistically insignificant.

However, in our study, we found that maximum number of cases are of exudative AMD (42.86%) followed by early AMD (33.34%) and 11.91% in intermediate and dry AMD each. This could be due to small sample size and unavailability of patients for examination until advanced visual loss has occurred due to low socioeconomic status of population enrolled.

Everekliogl u [14] found that decreased serum leptin levels were negatively correlated with disease severity, which was lower in late-ARMD (both dry and wet) patients than in the early-ARM subjects. Therefore, from the evidence stated above, decreased serum leptin levels might result in the loss of its lipidostatic function in cellular level and could cause an increased intracellular fatty acid accumulation within the lesions found in ARM or ARMD patients.

Seshasai *et al.* [2] conducted a population-based case–control study including Chinese and Indian adults aged 40–80 years. They reported that higher serum leptin levels were associated with a lower likelihood of AMD. This association was independent of traditional risk factors of AMD, including smoking, BMI, BP, and CVD. Mean levels of serum leptin among those with late, early, and without AMD were 8.8, 10.1, and 12.9 ng/mL.

However, in our study, we found maximum serum leptin level 2.58±0.99 ng/mL in exudative type followed by 1.06±0.99 ng/mL in the early ARMD and 0.50±0.08 ng/mL in intermediate ARMD followed by minimum 0.29±0.12 ng/mL in dry ARMD. The difference between serum leptin levels in different types of ARMD was statistically significant.

In the previous studies, there was constant decline in serum leptin concentration with disease progression. However, in our study, we found a decrease in serum leptin levels as it progressed from the early AMD to intermediate AMD and Dry AMD. However, the levels of serum leptin levels were higher in patients with exudative AMD.

CONCLUSION

Serum leptin levels were negatively correlated with the early, intermediate, and dry ARMD. However, we found these levels to be higher in exudative ARMD as compared to other stages. This may be due to some pathological process that is different in dry and exudative AMD. This can form the basis for further research in future. This study is one of its kind first study to evaluate the serum leptin levels in patients of ARMD in Indian subcontinent in best of our knowledge.

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AUTHORS' CONTRIBUTION

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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