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Original Article

A Comprehensive Evaluation of Serum Iron Status Indicators in Patients with Age-Related Macular Degeneration

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Abstract

Objectives: Iron is recognized as a significant contributor to oxidative damage, and its levels tend to rise with age, potentially worsening age-related diseases. The aim of this study was to investigate the role of serum iron metabolism markers in the pathogenesis of age-related macular degeneration (AMD).

Methods: The files of all AMD patients in Kocaeli University School of Medicine between January 2017 and March 2020 were reviewed retrospectively. By examining the files of AMD patients who applied to the eye outpatient clinic on the same dates, those dry AMD (dAMD) and neovascular AMD (nAMD) were recorded. As a control group, the records of patients without any AMD findings were obtained from the files of all patients who visited the clinic during the same time period. All records were recorded for analysis, including a comprehensive ophthalmological examination, laboratory data of fasting blood tests, and an internal medicine outpatient examination.

Results: Of the 164 participants, 50 were dAMD patients, 51 were nAMD patients, and 63 were patients non-AMD (control group). There was a significant difference between the groups' mean corpuscular volume (MCV), serum ferritin, and total iron-binding capacity (TIBC) ($p<0.050$). It was observed that the ferritin of those with AMD was significantly higher than the control group, whereas MCV and TIBC were found to be significantly lower ($p<0.050$). There was no significant difference in serum iron marker levels between nAMD and dAMD patients ($p>0.05$).

Conclusion: Assessing serum iron status indicators during the routine monitoring of AMD may provide insights into the associated risk profile of the condition.

Keywords: Age-related macular degeneration, serum ferritin levels, serum iron status

Introduction

Age-related macular degeneration (AMD) is a major cause of blindness worldwide, affecting more than 20% of the aging population (1). The major risk factors for AMD include cigarette smoking, nutritional factors, cardiovascular diseases, and genetic markers, including genes regulating complement (1). Dry AMD (dAMD) is a common manifestation of AMD, which is the leading cause of irreversible vision loss in individuals aged older than 50 years (1). At present, there is no proven drug treatment for dAMD, but several different treat-

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ment strategies are being investigated, including complement inhibition, neuroprotection, and visual cycle inhibitors (2). Oxidative stress has been implicated in the pathogenesis of dAMD (3). Neovascular AMD (nAMD) is a subtype of AMD characterized by the growth of abnormal blood vessels under the retina, which can leak fluid and blood, leading to scarring and irreversible damage to the macula (1). The primary mediator of angiogenesis in nAMD is reactive oxidative species, which upregulate the expression of vascular endothelial growth factor (4). nAMD typically develops later in the course of the disease and is more aggressive than the non-neovascular form of AMD.

Iron accumulation has been suggested as a factor in the development of AMD (5,6). It has been shown that serum ferritin levels increase with age and that AMD retinas have more iron within the photoreceptors, retinal pigment epithelium, and drusen than do age-matched control retinas (6,7). In mice deficient in the ferroxidase ceruloplasmin (Cp) and/or hephaestin (Heph), which are important for retinal iron homeostasis, retinal iron overload and degeneration with features of AMD were observed (5). Iron is necessary for numerous metabolic functions, yet it can also be harmful. Iron can significantly increase oxidative stress because it is a powerful producer of the hydroxyl radical, the most reactive of the free radicals (8). Oxidative stress is encouraged by iron dysregulation, which has been hypothesized as a pathogenic mechanism in neurodegenerative illness (9). Ferritin is a protein complex that is present in almost all living organisms (10). It plays a vital role in storing and releasing iron, which is an essential element for various physiological processes. Although ferritin is primarily known for its role in iron metabolism, recent research has highlighted its association with inflammation. Serum ferritin is an important inflammatory disease marker (10). Studies have shown that serum ferritin levels increase with inflammation (10-12).

There are limited studies evaluating the relationship between serum iron status indicators and patients with AMD (13-16). This study delved into examining potential connections between serum iron status markers and individuals affected by AMD, investigating these relationships and quantifying their significance. Furthermore, we quantified the strength of these relationships, which, to the best of our knowledge, represents the first measurement within a Turkish population.

Methods

Participants

This retrospective case–control study aimed to investigate the prevalence of dAMD and nAMD in patients who visited the eye outpatient clinic at Kocaeli University School of Medicine between January 2017 and March 2020. The study reviewed archive records of patients diagnosed with dAMD and nAMD using NUCLEUS XCE software. All records from clinic visits within the same timeframe were reviewed, and data from individuals without AMD symptoms were recorded to create a control group. The participants were divided into three groups: healthy participants, participants with dAMD, and participants with nAMD. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Kocaeli University School of Medicine's Local Ethics Committee (GOKAEK-2023/03.02).

Definition of AMD and Ophthalmic Examination

The patient's ophthalmologic examination notes were evaluated to determine their best corrected visual acuity, intraocular pressure measurements, slit lamp anterior segment biomicroscopic findings, and dilated fundus examination findings. The CR-2 AF retinal camera and Spectralis Heidelberg Retina Angiography + optical coherence tomography (OCT) records were examined, and color fundus photographs, OCT images, fundus fluorescein angiography, and indocyanine green angiography results were recorded. Based on these records, patients with AMD were categorized into two distinct groups: dAMD and nAMD (1).

Evaluation of Serum Iron Status Indicators

In this study, participants' laboratory parameters underwent examination both 1 month before and 1 month after their ophthalmic assessment. The assessment included serum iron status indicators such as ferritin level, iron level, total iron binding capacity (TIBC), and transferrin saturation. In addition, other laboratory parameters such as creatinine, white blood cells (WBC), mean erythrocyte volume (MCV), high-sensitivity C-reactive protein (hs-CRP) level, triglyceride (TGL), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were documented. These readings were obtained as "spot readings" on the same day, using blood samples drawn in the morning following a 12-h fasting period.

Exclusion Criteria

The ocular exclusion criteria for this study encompassed individuals who had undergone ophthalmic surgery or laser photocoagulation within 3 months preceding their inclusion. In addition, exclusion criteria involved the presence of nAMD in one eye and dAMD in the contralateral eye, along with individuals exhibiting chorioretinal disease, retinal branch or central vein occlusion, glaucoma, and pathologic myopia.

On a systemic level, exclusion criteria included patients with gastrointestinal conditions impacting iron levels and absorption, hypertension, anemia, diabetes, hematological disorders, malignancies, cardiovascular ailments, kidney disease, or creatinine levels exceeding 1.2 mg/dL. Other criteria

comprised active inflammatory conditions indicated by elevated hs-CRP and WBC counts, use of iron supplements or medications affecting serum iron levels, history of alcohol consumption and smoking, and a body mass index exceeding 30. These exclusion criteria were put in place to ensure that the study participants were representative of the general population and to minimize the potential confounding effects of other diseases or conditions on the study results.

Statistical Analysis

The statistical analysis employed SPSS version 25.0 for Windows (IBM Inc., Armonk, NY, USA). The Kolmogorov–Smirnov test assessed numerical data distribution. Descriptive statistics outline categorical variables in numbers and percentages, while numerical variables are expressed as mean±standard deviation. To compare multiple independent groups, the independent samples Kruskal–Wallis test was utilized. Utilizing the Bonferroni correction, validation was carried out. A $p<0.05$ was considered statistically significant. Binary logistic regression analysis was performed to determine the major risk factors affecting the presence of AMD.

Results

In this study, 164 participants were included, consisting of 79 (48.1%) men and 85 (51.9%) women with a mean age of 72 (range 62–82) years. The participants were divided into three groups: 63 healthy controls without AMD, 50 participants with dAMD, and 51 participants with nAMD. There was no significant difference in age and gender distribution between the three groups (respectively; p=0.504 p=0.818). The groups were also in agreement in terms of hs-CRP and WBC values, which are indicators of inflammation, and serum lipid levels (HDL, LDL, and TG) ($p>0.05$ for all). However, there was a statistically significant difference between the groups in terms of MCV, serum ferritin, and TIBC levels ($p<0.050$ for all). Pairwise comparisons of the groups showed that the levels of serum ferritin, MCV, and TIBC were significantly different between the non-AMD group and the groups with dAMD and nAMD. In the non-AMD group, the levels of MCV and TIBC decreased while the ferritin level increased. Nevertheless, there were not any notable statistical variations in serum MCV, ferritin, and TIBC levels observed between the nAMD and dAMD groups. Table 1 outlines the demographic characteristics of both groups along with serum iron and ferritin levels, transferrin saturation, TIBC, Hs-CRP, WBC, Hb, MCV, TGL, LDL, HDL levels, and creatinine levels. The P-values resulting from the pairwise group comparisons of laboratory parameters, which exhibited statistical differences among the three groups, are detailed in Table 2.

Table 1. Descriptive data and serum laboratory parameters of different groups

	non-AMD	dry-AMD	nAMD	p
n (%)	63 (38.41%)	50 (30.48%)	51 (31.09%)	
Age	71.53±8.76	73.41±5.75	73.08±8.20	0.504*
Male	29 (46%)	25 (50%)	25 (49%)	0.818*
Female	34 (54%)	25 (50%)	26 (51%)	
Creatinine (mg/dl)	0.83±0.13	0.82±0.17	0.87±0.29	0.954*
HDL (mg/dl)	53.87±12.84	56.01±14.99	53.51±14.12	0.360*
TGL (mg/dl)	124.44±53.47	130.11±64.94	141.34±62.05	0.379*
LDL (mg/dl)	130.01±35.06	121.23±27.00	127.34±36.15	0.456*
WBC ($\times 10^3/\mu L$)	6.98±1.60	7.52±1.81	7.19±1.83	0.141*
HsCRP (mg/dL)	2.43±1.12	2.96±3.74	2.90±2.82	0.541*
Hb (g/dl)	13.87±1.02	13.31±1.38	13.73±1.33	0.124*
MCV (fl)	91.56±3.45	80.29±10.32	84.42±5.05	≤0.001*
Serum ferritin level (ml/ng)	32.75±16.60	89.60±51.90	99.41±50.30	≤0.001*
Serum iron level (ug//dl)	89.52±27.40	84.00±38.16	78.70±30.02	0.076*
Total iron-binding capacity (μg/dL)	351.77±44.77	295.64±46.23	271.36±66.85	≤0.001*
Transferrin saturation (%)	0.25±0.08	0.25±0.11	0.32±0.14	0.198*

*Independent Samples Kruskal-Wallis test Bonferroni correction; AMD, age-related macular degeneration; nAMD, neovascular age-related macular degeneration; HDL, high-density lipoprotein; TG, triglyceride; LDL, low-density lipoprotein; HsCRP, high-sensitivity C-reactive protein; WBC, white blood cell; Hb, hemoglobin; MCV, mean corpuscular volume; Bold values are statistically significant outcomes with P-value <0.05; Mean±Std. Dev.

Table 2. The p values were determined by pairwise group comparisons of the laboratory parameters that were statistically different between the three groups

	MCV (fl)	Ferritin level (ml/ng)	Total iron-binding capacity (µg/dL)
non-AMD and dry-AMD	≤0.001	0.020	≤0.001
non-AMD and nAMD	≤0.001	≤0.001	≤0.001
dry-AMD and nAMD	1.000	1.000	0.931

*Independent Samples Kruskal-Wallis test Bonferroni correction; AMD, age-related macular degeneration; nAMD, neovascular age-related macular degeneration; MCV, mean corpuscular volume. Bold values are statistically significant outcomes with P-value <0.05.

The logistic regression analysis conducted in this study identified several risk factors associated with the presence of AMD. Table 3 shows that high serum ferritin, low mean MCV, and low TIBC levels were associated with an increased likelihood of developing nAMD compared to the non-AMD group. When all AMD groups were assessed combined, low MCV, low TIBC, high ferritin, and high transferrin saturation levels were found to increase the risk of developing AMD (Table 4).

Discussion

The research involved a comparison of serum iron status markers among dAMD, nAMD, and non-AMD patients, specifically those without anemia or active inflammation. The results showed that the presence of AMD increased serum ferritin levels and decreased MCV and TIBC. There was no significant difference in serum ferritin, MCV, and TIBC levels between the dAMD and nAMD groups. Serum iron levels were lower in the nAMD and dAMD groups compared to

Table 3. Binary logistic regression analysis between patients with nAMD and non-AMD groups

	Wald	p	OR	95%C.I for OR	
				Lower	Upper
MCV (fl)	18.917	0.000	0.557	0.428	0.725
Serum ferritin level (mL/ng)	6.999	0.008	1.048	1.012	1.086
Serum iron level (ug//dL)	0.039	0.844	1.007	0.943	1.075
Total iron-binding capacity (µg/dL)	4.045	0.044	0.976	0.952	0.999
Transferrin saturation (%)	0.358	0.550	0.943	0.779	1.142

* Binary logistic regression analysis. Bold values are statistically significant outcomes with P-value<0.05. nAMD: Neovascular age-related macular degeneration; OR: Odds ratio; CI: Confidence interval; MCV: Mean corpuscular volume.

Table 4. Binary logistic regression analysis between patients with AMD and non-AMD groups

	Wald	p	OR	95% C.I for OR	
				Lower	Upper
MCV (fl)	21.414	0.000	0.594	0.476	0.741
Serum ferritin level (mL/ng)	6.917	0.009	1.038	1.010	1.067
Serum iron level (ug//dL)	1.471	0.225	1.032	0.981	1.086
Total iron-binding capacity (µg/dL)	11.795	0.001	0.967	0.948	0.985
Transferrin saturation (%)	4.234	0.040	0.943	0.787	0.994

* Binary logistic regression analysis. Bold values are statistically significant outcomes with P-value<0.05. AMD: Age-related macular degeneration, OR: Odds ratio, CI: Confidence interval, MCV: Mean corpuscular volume.

the non-AMD group, but no statistically significant difference was observed. The logistic regression analysis unveiled a significant association between higher serum ferritin levels, along with lower MCV and TIBC levels, and the nAMD. The number of studies examining the relationship between serum iron indicators and dAMD and nAMD is limited (13-16). This pioneering study represents the first investigation into the association between serum iron status indicators and AMD specifically within the Turkish population.

Iron accumulation in the retina and iron-mediated oxidative stress are known risk factors for AMD, which is a leading cause of blindness (6,14,179. Studies have shown that the accumulation of iron in the human retina can cause typical AMD (6,18). Hereditary human diseases linked with systemic iron overloads, such as hemochromatosis and aceruloplasminemia, can also result in iron accumulation in the retina and AMD-like pathology, despite an intact blood-retinal barrier (6,19). In addition, the administration of intravenous iron for refractory anemias has been linked to retinal iron accumulation and an increased risk of AMD (19). Iron levels in the retina of elderly people were shown to be substantially higher than those of young adults, confirming the theory that iron plays a role in the pathogenesis of AMD (7). According to Ashok et al., (20) altered turnover of iron-loaded ferritin due to impaired lysosomal function is the cause of NaIO3-induced AMD in experimental models. The authors noted that increased iron in the AMD model was loaded with ferritin, leading to ferritin accumulation in vitro. This extracellular milieu is likely to become toxic to the nearby photoreceptors as a result of the release of these vesicles, leading to retinal disease that mimics AMD.

The relationship between serum iron markers and AMD is still a topic of debate in the literature, and there is no clear consensus on this issue. Richer et al. (14) conducted a study in 2002 to evaluate iron overload serum markers and dietary intake in patients with atrophic AMD. The authors concluded that evaluating dietary or serum systemic iron status biomarkers at a single clinical visit is unnecessary. However, they suggested that serum ferritin measurements may be more valuable in the pathogenesis of AMD. Wysokinski et al. (15) did not find any correlation between serum iron levels and the development of AMD, although the level of soluble transferrin receptor was much higher in AMD patients compared to controls. On the other hand, serum ferritin levels were linked to an early form of AMD and had a close relationship with traditional risk factors for AMD, according to Oh et al. (13) Čolak et al. (16) found that the antioxidant defense system and serum ferritin capacity were significantly reduced in AMD patients. These studies suggest that the relationship between serum iron markers and AMD may depend on various factors, such as the type and stage of AMD, as well as other risk factors for the disease. In our study, we found that serum ferritin levels were significantly higher in patients with AMD compared to the control group. In addition, TIBC levels were significantly lower in patients with AMD than in the control group. The results of the logistic regression analysis demonstrated a notable association between elevated serum ferritin levels and reduced TIBC levels with the nAMD.

MCV levels were found to be significantly lower in the AMD group compared to the healthy control group, indicating a potential association between low MCV levels and AMD development. It is widely acknowledged that younger red blood cells typically exhibit a higher MCV. However, an investigation into oxidative damage in rats post-intense exercise revealed a notable but slight decrease in MCV (21). This points toward that the decreased MCV levels observed in AMD might be linked to oxidative damage in the development of the disease. To the best of our knowledge, our study is the first to evaluate the relationship between AMD development and serum MCV levels.

The present study encounters certain limitations in result interpretation. First, due to the study's limited sample size and its retrospective, cross-sectional nature establishing a causal relationship between serum iron indicators and AMD was impeded. Comprehensive prospective studies and meticulous analyses are essential to affirm the findings and unravel the underlying mechanisms. In addition, the study was conducted at a singular center, focusing on laboratory parameters derived from patients within a specific period in the internal medicine department. This restricts the generalizability of the findings. Subsequent multicenter cohort studies are required to authenticate the results and determine their applicability to a wider spectrum of AMD patients.

Conclusion

These findings suggest that serum ferritin, MCV, and TIBC levels may be useful biomarkers for identifying individuals at risk of developing AMD. Moreover, to the best of our knowledge, we have identified a novel association between AMD and reduced MCV levels. However, further studies are needed to confirm these findings and to determine the clinical utility of these biomarkers in the diagnosis and management of AMD.

Disclosures

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and was approved by the Kocaeli University School of Medicine's Local Ethics Committee (GOKAEK-2023/03.02).

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