ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 01 | January - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SERUM LEVELS OF 25-HYDROXY VITAMIN D, C-REACTIVE PROTEIN AND FERRITIN: PROMISING BIOMARKERS FOR AMD RISK

Biochemistry

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ABSTRACT

Background: Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in individuals over 55, particularly in developing countries. This multifactorial disease affects the macula and is influenced by genetic and environmental factors. This study explored the role of serum 25-hydroxy vitamin D, C-reactive protein (CRP), and ferritin as potential biomarkers of AMD risk and progression.**Methods:** A cross-sectional study was conducted on 120 AMD patients at a tertiary care hospital in Central India. Participants were classified into early, intermediate, and advanced AMD stages. Serum levels of 25-hydroxy vitamin D, CRP, ferritin, and lipid profiles were analysed using ANOVA and Pearson correlation. **Results:** Advanced AMD cases had significantly lower 25-hydroxy vitamin D levels and higher CRP and ferritin levels compared to earlier stages. Negative correlations were observed between 25-hydroxy vitamin D and both CRP ($r = -0.899$, $p < 0.001$) and ferritin ($r = -0.578$, $p < 0.001$). A positive correlation was noted between CRP and ferritin ($r = 0.605$, $p < 0.001$). **Conclusion:** Serum levels of 25-hydroxy vitamin D, CRP, and ferritin are significantly associated with AMD severity, highlighting their potential as biomarkers for disease progression and risk assessment.

KEYWORDS

Age-related macular degeneration, 25-hydroxy vitamin D, C-reactive protein, Ferritin, Biomarkers, Disease progression

INTRODUCTION:

Age-related macular degeneration (AMD) is a complex disease associated with a high risk of complications that affect vision[1]. It develops mainly in people over 50 years of age and a key characteristic feature is the accumulation of a series of extracellular deposits in the macula, known as drusen. It is the main cause of irreversible blindness in patients over 55 years of age[2].

Iron is vital for cells and various physiological functions, but a lack of iron sequestering proteins like ferritin and improper accumulation of this metal can lead to harmful reactive oxygen species. This process is linked to the onset and development of inflammatory responses and neurodegenerative diseases[3].

Moreover, the increased appearance of ferritin in extracellular spaces may be the result of extensive cell damage[4].

AMD has also been linked to 25- Hydroxy Vitamin D deficiency, suggesting a potential role in its pathogenesis. Inflammation plays a crucial role in AMD, supported by the presence of inflammatory markers[5].

C-reactive protein (CRP) stands out as a sensitive, yet nonspecific inflammation marker linked to various chronic conditions, including AMD. This connection is reinforced by the presence of complement factors in AMD-related drusen, suggesting a role for local inflammation in disease development. Studies have shown that elevated CRP levels are associated with an increased risk of AMD, highlighting its potential as a valuable marker in understanding AMD pathogenesis and systemic immune activation[6].

Present study aims to assess the levels of serum 25-Hydroxy Vitamin D, Ferritin, and CRP in patients with different stages of AMD, and to evaluate their potential as biomarkers for disease risk and progression.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Biochemistry in collaboration with the Department of Ophthalmology at Gandhi Medical College and Hamidia Hospital, Bhopal, India, from August 2022 to February 2024.

The study protocol was approved by the Institutional Ethics Committee (approval number: 32226/MC/ IEC/2022), and written informed consent was obtained from all participants before enrollment.

A total of 120 patients diagnosed with Age-related Macular Degeneration (AMD) were included in the study. Participants were selected based on the following criteria: age 40 years and above, and diagnosis of AMD by indirect ophthalmoscopy, confirmed by optical coherence tomography. Patients with other retinal diseases, history of retinal surgery, or systemic diseases that could affect biomarker levels were excluded.

Based on the severity of macular changes, patients were classified into three groups by experienced ophthalmologists: early AMD (n=39), intermediate AMD (n=55), and advanced AMD (n=26), following the Age-Related Eye Disease Study (AREDS) criteria.

Venous blood samples (5 mL) were collected from each participant after an overnight fast. Samples were allowed to clot for 1 hour at room temperature and then centrifuged at 3000 rpm for 15 minutes to separate the serum. Serum 25-hydroxy Vitamin-D was measured by Chemiluminescence Immunoassay (CLIA) method. Serum ferritin was analyzed by immunoturbidimetric assay using a fully automated biochemistry analyzer (Beckman Coulter DxI800).

Serum CRP was measured by immunoturbidimetric assay using a fully automated biochemistry analyzer (Biosystems BA200). All assays were performed according to the manufacturers' instructions, with reference ranges as follows: 25-Hydroxy Vitamin D (20-50 ng/mL), Ferritin (20-250 ng/mL for males, 10-120 ng/mL for females), and CRP (< 5 mg/L). Quality control samples were run with each batch of patient samples to ensure assay reliability.

Statistical analysis was performed using Epi-info software. Categorical variables were expressed as numbers and percentages, while continuous variables were presented as mean ± standard deviation.

Data were analyzed using ANOVA, and relationships between dependent variables were assessed using Pearson's correlation. Associations between dependent variables and AMD stages were examined through regression analysis. A p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The majority of patients were in the 50-59 years age group (31.7%), with a female predominance (60.0%). AMD severity was stratified into early (32.5%), intermediate (45.8%), and advanced (21.7%) stages.

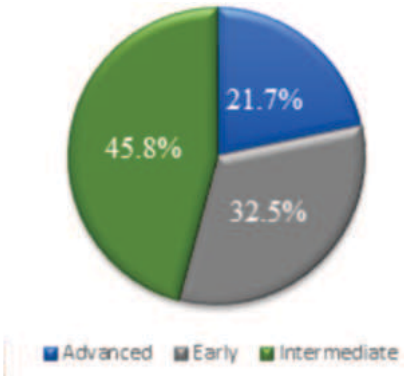

Figure-1: Graphical presentation stage-wise distribution of the cases

Table: Mean levels of Serum 25-Hydroxy Vitamin D, Ferritin and CRP among stages

Stages	Early	Intermediate	Advanced	p-value
No. of Cases	39	55	26	-
Serum 25-Hydroxy Vitamin D (ng/mL)	24.33 ± 1.89	18.86 ± 1.32	11.49 ± 1.22	<0.001*
Serum Ferritin (ng/mL)	174.55 ± 37.75	209.59 ± 24.92	240.03 ± 26.77	<0.001*
Serum CRP (mg/L)	12.43 ± 4.30	30.66 ± 4.48	51.33 ± 4.54	<0.001*

[*Statistically Significant]

The means of the 25-Hydroxy Vitamin D levels in the early, intermediate, and advanced shows a decreasing trend ($p<0.001$). Serena et al., 2022 also reported that those with AMD had significantly lower levels of serum 25-Hydroxy Vitamin D compared to those without AMD[1]. In a study, Millen AE et al., 2011 found that higher levels of 25-Hydroxy Vitamin D were associated with a reduced risk of early AMD in women[7]. Another study by Parekh et al., 2007 found that low levels of 25-Hydroxy Vitamin D were associated with an increased risk of advanced AMD in older adults[8]. The observed decrease might be attributed to antioxidant and anti-inflammatory properties of vitamin D, which could play a protective role in AMD pathogenesis. As the disease progresses, lower vitamin D levels may contribute to increased oxidative stress and inflammation in the retina.

The means of the ferritin levels also shows an increasing trend with the progression of the disease ($p<0.001$). In a study, Oh et al., 2016 investigated the relationship between ferritin and AMD in a Korean population, finding that higher serum ferritin levels were associated with an increased risk of early AMD[3]. The increasing ferritin levels may indicate iron overload in the retina as AMD progresses. Excess iron can promote oxidative stress through the Fenton reaction, potentially contributing to retinal damage and disease advancement.

There were statistically significant differences among the means of CRP levels in the early, intermediate, and advanced cases of AMD ($p<0.001$). Studies have shown that elevated levels of CRP are associated with an increased risk of AMD. Hong et. al., 2011 in a meta-analysis of 11 studies, CRP levels were found to be significantly higher in individuals with AMD compared to those without AMD[9]. Another systematic review and meta-analysis of 14 studies by Molins et. al., 2018 found that elevated CRP levels were associated with an increased risk of AMD[6].The increasing CRP levels likely reflect a growing systemic inflammatory response as AMD progresses. Chronic inflammation is a known contributor to AMD pathogenesis, potentially damaging retinal tissues and exacerbating disease progression.

The levels of serum CRP and 25-Hydroxy Vitamin D significantly correlated with one another ($r = -0.899$, $p<0.001$). Similarly, Hernández-Álvarez et al., 2019 revealed a significant inverse correlation between 25-Hydroxy Vitamin D levels and CRP, indicating a potential relationship between these biomarkers in the context of AMD[10].

Similarly, the relationship between ferritin and 25-Hydroxy Vitamin D levels demonstrated a significant negative association ($r = -0.578$, $p < 0.001$). Further research focusing on the specific relationship between serum ferritin and 25-Hydroxy Vitamin D levels in AMD patients could provide valuable insights into their potential roles in AMD pathogenesis or progression.

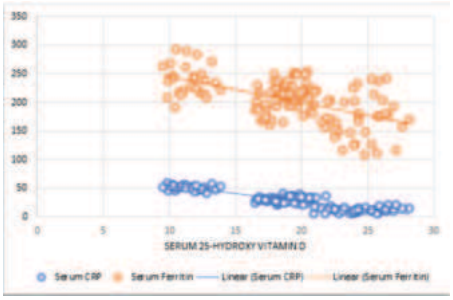

Figure-2: Scatterplot diagram of correlation between serum ferritin and CRP with 25-Hydroxy Vitamin D in patients of AMD

Regression analysis demonstrated strong associations between serum biomarkers and AMD stage. Serum 25-hydroxy vitamin D ($R^2=0.897$, $p<0.001$) and CRP ($R^2=0.910$, $p<0.001$) showed particularly strong relationships with AMD stage, explaining 89.7% and 91% of the variability, respectively. These results highlight the potential of these biomarkers as predictive tools for AMD progression.

CONCLUSION:

The analysis of 25-Hydroxy Vitamin D levels across different stages of AMD revealed a decreasing trend, collaborating previous findings associating higher levels with reduced risk in early stages and increased risk in advanced stages. Ferritin, investigated as a potential biomarker, exhibited an increasing trend with early AMD risk, highlighting its significance in disease detection. However, CRP levels showed significant differences across AMD stages, aligning with existing literature linking elevated CRP levels with AMD risk. These findings emphasize the utility of 25- Hydroxy Vitamin D, ferritin, and CRP as potential biomarkers for AMD risk assessment and disease progression monitoring.

Surprisingly, despite variations in CRP, ferritin, and 25-Hydroxy Vitamin D levels across different disease stages, our study did not reveal significant correlations among these biomarkers. This highlights the complexity of the biochemical interplay in AMD and implies that these markers may independently contribute to the disease's multifaceted pathogenesis.

Further research is warranted to search into the important interactions of these biochemical factors and their potential implications for understanding and managing the complexities of AMD.

Limitations Of The Study:

The study's limitations include a sample size of 120 patients from a specific geographic location, potentially limiting the generalizability of findings to broader populations. Conducted at a single center, the research might introduce bias and restrict the diversity of patient characteristics and disease manifestations. Additionally, focusing on serum biomarker levels at a single instance ignores potential fluctuations due to diurnal variations or short-term dietary changes. Furthermore, the exclusion of potential biomarkers like genetic markers or oxidative stress indicators, despite standard methods, may impact the comprehensiveness of the study's findings.

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