Serum C – reactive protein level and other risk factors in age related macular degeneration patients

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Abstract

Introduction: Recent evidence suggests that inflammation and abnormalities of innate immunity play a role in the pathogenesis of age-related macular degeneration. In light of the evidence linking inflammation and AMD, it is of interest to determine whether CRP levels and other markers of inflammation are predictive of AMD.

Methodology: 50 patients of AMD who satisfy the inclusion and exclusion criteria were included by non-random purposive sampling method. Relevant investigations like FFA and OCT were done. Serum CRP levels were assessed by latex agglutination by SeroCHEK AGAPPE Kit having analytical sensitivity as 6mg/L.

Results: Majority of study patients have early AMD changes (70%) as compared to late ARMD changes (30%).Wet AMD corresponds to 16% of all the study patients. Both the groups, early as well as late AMD patients have male preponderance. Majority of patients with early AMD have vision from 6/6 to 6/12 and those with late ARMD have vision 6/60 and less than 6/60. Patients with hypertension, diabetes mellitus, cardiovascular disorders and smokers found no relationship with AMD. In the present study, maximum number of AMD patients have pseudophakia. All the study patients with early as well as late AMD changes, have serum CRP levels below 6mg/L i.e; below the reference value taken in this study.

Conclusion: The present study does not support the theory alleging non-specific systemic inflammation in the aetiology and natural history of age related macular degeneration. Though, this may be due the small study sample.

Keywords: CRP, AMD, Inflammation.

Introduction

Age-related macular degeneration (AMD) is the most common form of legal blindness in most of the developed countries.[1] Globally, AMD ranks third as a cause of blindness after cataract and glaucoma according to WHO. The World Health Organization (WHO) estimated in 2002 that 8.7% of the world's blindness was due to AMD. Recent evidence suggests that inflammation and abnormalities of innate immunity play a role in the pathogenesis of age-related macular degeneration (AMD).[2] A strong association between a common variant of the gene for complement factor H (CFH) and AMD has recently imparted considerable weight to this hypothesis.[3] Patterns of AMD progression viewed in the context of the development of an increasingly proinflammatory status with age suggest the possibility that lesions and/or geographic atrophy. In the case of neovascular AMD, this process involves an overt inflammatory/neovascular response originating from the choroidal vasculature.[7,8] Circulating levels of C-reactive protein (CRP) have been intensively studied, and a single measure reliably indicates the degree of underlying systemic inflammation in asymptomatic adults. In light of the evidence linking inflammation and AMD, it is of interest to determine whether CRP levels and other markers of inflammation are predictive of AMD. Thus, the present study was undertaken to determine the association between AMD and serum CRP levels.

Methodology

The ethical and research committee of our hospital approved this study. Informed verbal consent was obtained from each participant. This study was conducted between January, 2014 to January 2015.

Type of study: Descriptive study

Sampling method: Purposive sampling method.

Study population: The study population comprised of ARMD patients aged 60 years and older fulfilling inclusion criteria that visited our institution during the study period for their eye ailments.

Inclusion criteria:

1) All clinically diagnosed ARMD patients of more than or 60 years of age.

Exclusion criteria:

1) AMD patients on prior treatment (antioxidants and anti-VEGF).
2) AMD patients with other retinal diseases affecting macula.
3) AMD patients with diagnosed hepatic diseases.
4) AMD patients with history of any acute inflammatory disease presently or 3-4 days in the past.

Sample size: Estimated to be 50 cases with formula

$$S = Z^2pq/d^2$$

Where p (prevalence) = 3.6%, d (precision) = 5%, maximal allowable error is 5% and power of study is 80%.

Data are collected using a piloted proforma meeting the objectives of the study by means of personal interview with the patients. Detailed case history including personal and family history is taken and comprehensive systemic and ocular examination including visual acuity, refractive status and slit-lamp biomicroscopy using 78D is done. To assess distant vision Snellen's illiterate "E" Fig., held at 6 m distance from the patient is used. Near vision is assessed using Snellen near vision Fig.

Assessment of AMD: Diagnosis of AMD patients is made using direct ophthalmoscopy and using slit lamp biomicroscopy. Fundus photographs are taken. Fundus fluorescein angiography and Optical coherence tomography has been done for AMD cases not showing typical geographical atrophic pattern and also done for all wet AMD patients. Once the diagnosis of AMD is established, patients are included in study group and eye having more AMD changes of the two is used for analysis. Complete blood count and liver function test are done and patients having values within normal limits are included. Classification by International Epidemiological Age-related Maculopathy Study Group is followed for diagnosis and classification of AMD in this study. Metamorphopsia and central scotoma are assessed using Amsler grid Fig. 1. Then early AMD patients are treated by anti-oxidants and advanced and treatable AMD patients are referred to vitreo-retinal surgeon.

Assessment of risk factors: Detailed medical history including hypertension, diabetes mellitus and cardiovascular diseases are taken. Cigarette-smoking and family histories are also taken.

Measurement of serum c-reactive protein: 2ml of venous blood sample is used for serum CRP analysis by latex agglutination by Sero CHEK AGAPPE kit having analytical sensitivity as 6 mg/L with diagnostic sensitivity 95.6% and diagnostic specificity as 96.2% in the same laboratory settings.

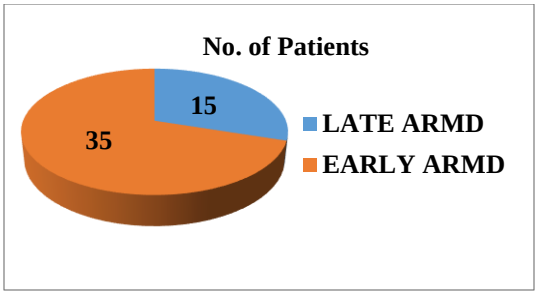


Fig. 1: Percentage of early and late ARMD

Results

1. Majority of cases of early AMD (74.2%) are in the age group 60-70 years. Among late AMD patients, proportion of patients with late AMD increases as age advances.
   Results obtained from Table 1 are not statistically significant (p>0.05).

Table 1: Age distribution among patients

Age in years	Early AMD	Late AMD	Total
60-65	18	4	22
66-70	8	4	12
71-75	5	1	6
76-80	3	4	7
81-85	1	2	3
	35	15	50

2. Majority of the study patients have early ARMD (70%) as compared to late AMD (30%). Wet AMD corresponds to 16% of the total AMD patients.
3. Fig. 2 depicts that majority of patients belong to rural population- Early AMD (77%) and Late AMD (73%) but result is not statistically significant (p>0.05).

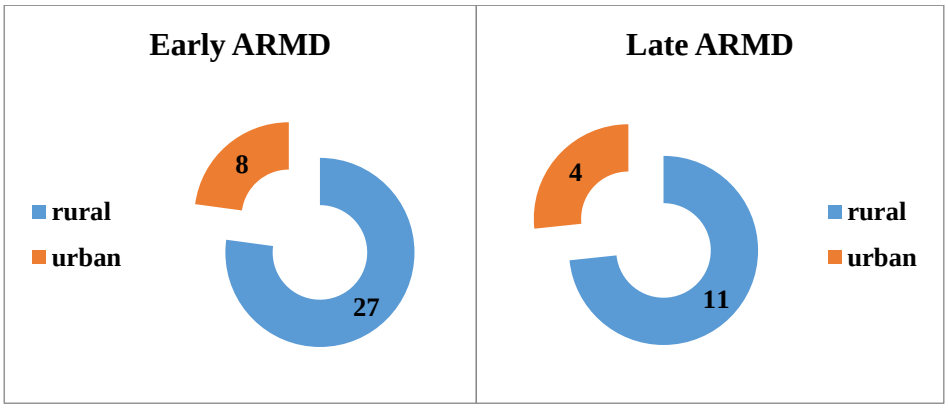


Fig. 2: Demographic profile of patients

4. Fig. 3 shows that both groups i.e; early and late ARMD have male preponderance-Early AMD (60%) and Late AMD(73%).

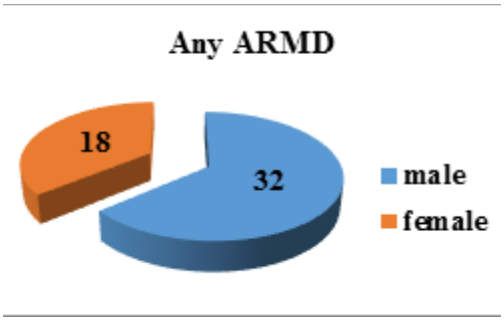
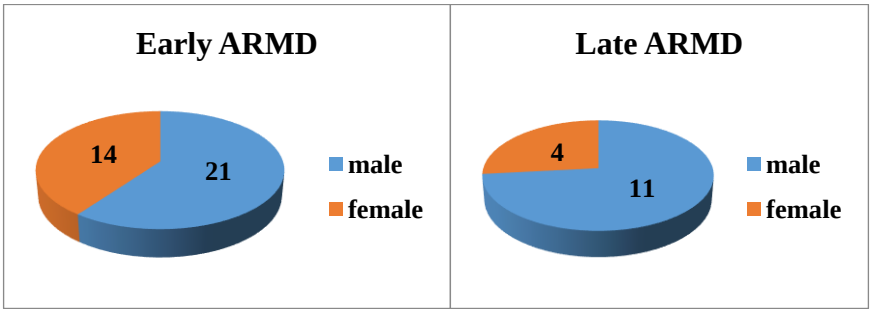


Fig. 3: Sex distribution among patients



5. Results of Table 2 depicts that 68.5% of Early AMD patients are hyprtensive while 53.3% of Late AMD patients are hypertensive but the relationship between hypertension and AMD is not statistically significant (p>0.05).

Table 2: Relationship between hypertension and ARMD

Hypertension	Early ARMD	Late ARMD	Total
Present	24	8	32
Not present	11	7	18
	35	15	50

6. Table 3 depicts that 42.8% of Early AMD patients have Diabetes mellitus while 26.7% of Late AMD have Diabetes mellitus but there is no significant relationship between diabetes mellitus and AMD.
7. Table 4 depicts that 83% of Early AMD patients does not have cardiovascular disease while 93.3% of Late AMD patients do not suffer from any cardiovascular problems but significant correlation (p>0.05) was not found between cardiovascular diseases and AMD patients in the present study.

Table 3: Relationship between diabetes mellitus and ARMD

Diabetes Mellitus	Early ARMD	Late ARMD	Total
Present	15	4	19
Not present	20	11	31
	35	15	50

Table 4: Relationship between cardiovascular diseases and ARMD

CVS Disorders	Early ARMD	Late ARMD	Total
Present	6	1	7
Not Present	29	14	43
	35	15	50

8. Fig. 4 depicts that 53% of Late AMD patients are smokers but 74% of Early AMD are non-smokers but no significant correlation (p<0.05) is found between smoking and AMD in this study.

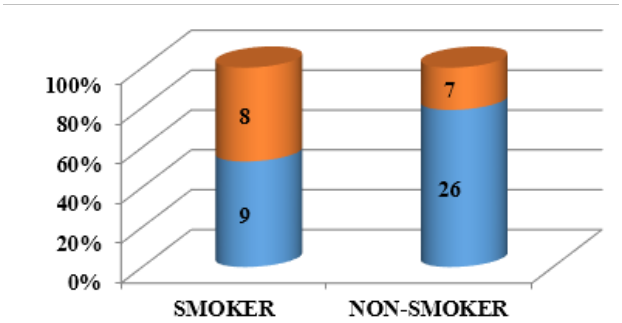


Fig. 4: Relationship between smoking and early and late ARMD

9. Fig. 5 depicts that majority of patients with early AMD have vision from 6/6 to 6/12(58% of all the AMD patients) and majority of patients with late AMD have vision 6/60 and less than 6/60. Patients with vision less than 6/60 correspond to 16% of all the AMD patients. Out of the patients having vision less than 6/60 in the worst eye (8 patients), 4 patients (8% of total AMD patients) have vision less than 6/60 even in the other eye. More severe vision loss is typically associated with wet ARMD.

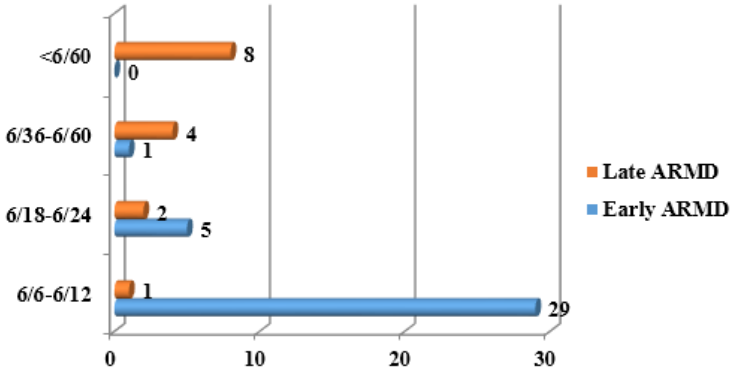


Fig. 5: Relationship between BCVA and ARMD

10. Table 5 depicts that among all the AMD patients, maximum patients with early as well as late AMD have pseudophakia. 42.8% out of all early AMD patients have pseudophakia and 40% out of all late AMD patients have pseudophakia. Next is nuclear sclerosis. 12 out of 50 patients (have nuclear sclerosis).

Table 5: Relationship between status of lens and ARMD

Lens	Early ARMD	Late ARMD	Total
Clear	12	3	15
PSC	1	1	2
NS1	2	1	3
NS2	3	2	5
PSC+NS1	2	2	4
Pseudophakia	15	6	21
	35	15	50

11. All patients with early AMD as well as late AMD have negative serum CRP levels (<6mg/dl).

Discussion

The cause of AMD is multifactorial and influenced by age, ethnic background, and a combination of environmental and genetic factors.[7]

Family history is an established determinant of risk as best shown from twin studies.[7] The importance of heredity was confirmed when polymorphisms within genes encoding complement factor H, complement factor B, and complement factor C3 were shown to be predictors of risk for AMD. While it is unknown how mutations in the complement cascade predispose patients to AMD, it is now accepted that dysregulation of the innate complement pathway leads to aberrant inflammatory responses resulting in the accumulation of debris within Bruch's membrane; however, the mechanism whereby the complement pathways initially becomes activated remains to be elucidated. Perhaps, a wide range of inflammatory stimuli over a lifetime may be responsible resulting in this predilection to develop the signs and symptoms of AMD.

Other risk factors for the development of AMD have been identified despite a limited understanding of the exact pathophysiology. Various researchers implicate atherosclerosis, oxidative damage, phototoxicity, inflammation, and diet.[8] Systemic arterial hypertension

and cigarette smoking are associated with an increased risk of neovascular AMD.

In the present study, 50 cases of AMD of 60 years and above were studied and mean age of all the patients was 67.54 years. Mean age for females in the study was 68.33 years and for males was 69.25 years.

Mean age for patients with early AMD was 67.43 years and that with late AMD was 72.57 years. The present study also shows that as age advances, number of patients with late AMD also increases which is similar to the result found in Andhra Pradesh Eye Disease Study done by Krishnaiah S, Das T, Nirmalan PK, et al. in 2005[9] which had reaffirmed age as a significant risk factor for AMD and in multivariate analysis showed that the adjusted prevalence of AMD was significantly higher in those aged 60 years and above. The Aravind Comprehensive Eye Study by Nirmalan PK, Katz J, Robin AL et al. in 2004[10] had similar findings: prevalence of early and late AMD rose significantly with increasing age.

The role of gender in AMD is contentious. Although some studies have found the female gender to be associated with a greater prevalence of AMD, possibly confounded by longer life expectancy and increased health care utilization, other studies like "Age related macular degeneration: aetiology, pathogenesis and therapeutic strategies" by Ambati J, Ambati BK, Yoo SH, et al[11] have revealed no gender differences in AMD risk which is similar to the finding seen in our study. In India, neither the Andhra Pradesh Eye Disease Study done by Krishnaiah S, Das T, Nirmalan PK, et al. in 2005[9] nor Aravind Comprehensive Eye Study by Nirmalan PK, Katz J, Robin AL et al. in 2004[10] found any association between gender and AMD.

Cardiovascular risk factors have long been purported in the pathogenesis of AMD.[8,11] Of these, systemic hypertension is known to be associated with choroidal neovascularization according to Age-related Eye Disease Study Research Group 2000. However, studies in India have not found such a relationship which is in accordance with our study. Although the Andhra Pradesh Eye Disease Study done by Krishnaiah S, Das T, Nirmalan PK, et al. in 2005[9] revealed higher odds for AMD in hypertensive subjects compared with normotensive subjects, the difference was not statistically significant. Similarly, the "Aravind Comprehensive Eye Study" done by Nirma./lan PK, Katz J, Robin AL, et al. in 2004[10] did not find a statistically significant association between the two.

The Beaver Dam Study[12] showed trends of association of hyperglycaemia to exudative AMD in men but the present study has shown no significant association between AMD and diabetes mellitus.

Smoking is a consistent risk factor for AMD and blindness according to" Age related macular degeneration: aetiology, pathogenesis and therapeutic strategies" by Ambati J, Ambati BK, Yoo SH, et al[11] which is also found in our study but the result is not statistically significant.

In this study, 93% has bilateral disease as compared to the study "Prevalence and determinants of Age Related Macular Degeneration in 50 years and older population: A hospital based study in Maharashtra, Pune" by Kulkarni SR, Aghashe SR, Deshpande MD, et al[13] in which bilateral disease was seen in 70.8% of AMD patients and 29.2% had unilateral disease. The same study also proposed the percentage of early AMD to be 82.6% and late AMD to be 17.4%. This is similar to our study where we found that early AMD accounts for 70% and late AMD for 30%.

In this study, we found that wet AMD corresponds to 16% of the total AMD patients which is similar to most of the studies. Patients with BCVA <3/60 corresponds to 8% in the present study as compared to 4.2% seen in "Prevalence and determinants of Age Related Macular Degeneration in 50 years and older population: A hospital based study in Maharashtra, Pune" by Kulkarni SR, Aghashe SR, Deshpande MD, et al.[13]

According to the study "Comparison of visual acuity in Macular degeneration patients measured with Snellen and Early Treatment Diabetic Retinopathy Study Fig.s by Falkenstein IA, Cochran DE, Freeman WR, et al,[14] 77% patients had vision poorer than 6/9 and 23% had vision better than 6/9. Majority of patients had vision between 6/36 to 6/12. Very less patients had vision less than 6/60. This is similar to the present study, in which majority of patients (38%) have vision between 6/36 to 6/12 and only 8% patients have vision less than 6/60. More severe vision loss is typically associated with wet ARMD.

In the present study, majority of AMD patients were pseudophakic (42%). Among early AMD patients, 42.9% were pseudophakic and among late AMD patients, 40% were pseudophakic. In several previous studies, a history of cataract surgery has been found to be associated with an increased risk for advanced AMD. Investigators have postulated that this association might arise because the cataractous lens can block damaging ultraviolet light. Inflammatory changes after cataract surgery may also cause progression of early to late AMD. Next in percentage was clear lens which was found in 31.4% of early AMD patients and 20% of late AMD patients.

Out of AMD patients who have cataract, nuclear sclerosis (24%) was more common than cortical cataract and posterior subcapsular cataract. This is similar to the BDES finding, in which photographs of the lens and macula were graded and nuclear sclerosis was associated with increased odds of early AMD but not of late AMD. Neither cortical nor posterior subcapsular cataracts were related to AMD.[15]

Serum CRP levels were below the reference value (<6mg/L) in all the AMD (early as well as late) patients in the present study. The same finding was found in the studies done by McGwin G, Hall TA, Xie A, et al(2005),[16] Klein R, Klein BE, Knudtson MD, et

al(2005),[17] Boey P Y et al (2010)[18] and Klein R, Klein BE, Marino EK, et al.(2003).[19]

All the above mentioned studies, showed no association between CRP and AMD. These data do not support the theory alleging non-specific systemic inflammation in the aetiology and natural history of this disease. Though, this may be due the small study sample. Thus, further study with larger study sample is required to establish the exact relationship between serum CRP levels and AMD.

Conclusion

In the present study, no statistically significant positive relationship was noted between serum CRP levels and the AMD patients i.e; serum CRP level in AMD patients (early as well as late AMD) was not found to be elevated above the reference value (6mg/L) taken in this study.

This may be due to the fact that the retina makes up an extremely small part of the body and that the portion involved in AMD is even smaller, the local inflammatory reaction reported to exist therein by laboratory studies would not be expected to create detectable levels of acute phase reactants in the peripheral blood. Moreover, the blood retinal barrier may also play a role in restricting the detection of markers of inflammation in the peripheral circulation.

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