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Analysis of Biomarkers (Homocystine/CRP) as Risk Factors for Age-Related Macular Degeneration: A Case Control Study

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Abstract

Introduction: *Age Related Macular Degeneration (AMD) is a progressive disorder of the macular area that becomes clinically apparent usually after 50 years of age. When it progresses to advanced stages it can result in severe loss of central vision with significant impact on quality of life. Age-related macular degeneration is the leading cause of irreversible blindness in the western world. With the shifting of population distribution to older age groups, the prevalence, effect, and healthcare cost of AMD is expected to increase substantially*

Purpose: *To measure and contrast biomarkers (high-sensitivity C-reactive protein (hsCRP) and homocysteine (Hcy)) in individuals with age-related macular degeneration (AMD) and control individuals without AMD*

Design: *Case–control study*

Participants: *30 AMD Cases and 30 Healthy Subjects who attended Eye department of Tertiary hospital.*

Methods: *Both affected and unaffected individuals underwent testing for hsCRP and homocysteine.*

Main Outcome Measures: *Mean hsCRP and homocysteine levels in affected and unaffected individuals.*

Results: *Mean hsCRP levels for affected and unaffected individuals were 7.107 and 2.10 mol/l, respectively (P value < 0.01; CI 95%).Mean homocysteine levels for affected and unaffected individuals were 15.89 and 9.67 mol/l, respectively (P value < 0.01; CI 95%). In logistic regression models, elevated hsCRP, and homocysteine were risk factors for AMD. There were no significant differences between cases and controls in terms of Age, gender, Diabetes and Hypertenson.*

Conclusion: *Elevated levels of hsC-reactive protein and Homocysteine levels are associated with an increased risk of age-related macular degeneration*

Keywords: *ARM, Homocysteine, hsCRP, Inflammation.*

Introduction

Age-Related Macular Degeneration (AMD) is the leading cause of legal blindness in the Western World in persons older than 60 years of age.[1] It is a complex multifactorial disease whose pathogenesis is poorly understood. It has two main presentations, dry and wet. Although the dry form, which is slowly progressive, accounts for 90% of all cases, the Wet AMD form accounts for 88% of all cases of blindness attributable to AMD.[1,2]

Numerous epidemiological studies like The Eye Disease Case–Control Study,[2] The Age-Related Macular Degeneration Risk Factors Study Group,[3] have implicated an interaction of genetic and environmental factors in the pathogenesis of AMD and have tried to elucidate modifiable environmental risk factors like Smoking, Hypertension,[3] obesity,[4,5] Increased cholesterol [2] and elevated plasma fibrinogen[5]for Age related macular degeneration.

Wet AMD involves a degenerative process that leads to proliferation of choriocapillaries into the subretinal space.[6] Several authors have recently reported increased levels of plasma vascular endothelial growth factor, von Willebrand factor, and fibrinogen, as well as increased plasma viscosity in patients with AMD. These findings suggest an association of markers of angiogenesis, hemostasis, and endothelial dysfunction with AMD,[6] and are supported by the Rotterdam study, which reported an association between AMD and atheriosclerosis,[7] for which elevated plasma Homocysteine levels is apparently an independent risk factor.[8]

There is mounting evidence from laboratory based studies that inflammation plays a key role in the pathogenesis of AMD.[9] The inflammatory marker CRP has recently been shown to be an independent risk for cardiovascular and peripheral arterial disease and a pathogenic factor leading to endothelial dysfunction in the cell culture model.[10]. Moreover, elevated concentration of CRP has been associated with an increased risk for hypertension.[11]. Because hypertensionis considered a major risk factors for retinal vascular disorders, their association with inflammation and endothelial dysfunction has been suggested in humans with retinopathy.[12]Atherosclerosis is a known risk factor for AMD, most likely through decreased choroidal blood flow, directly or indirectly impairing the functioning of the RPE,[13] and is also associated with elevated hsCRP concentration, which may contribute to the higher risk of AMD.[14]

On the basis of these findings, we could hypothesized that AMD may also be associated with elevated plasma levels of homocysteine, and hsCRP.

Material and Method

After approval from Institutional scientific committee and ethical committee. A Case-Control, one time study was performed on 30 AMD Cases and 30 Healthy Subjects after obtaining a proper signed information consent in their regional language.

Study participants included 30 AMD affected individuals and 30 healthy unaffected individuals who attended Eye department of tertiary hospital, aged 50 to 90 years. Theywere adjusted for, Age, sex and environmental factors.

An extensive medical and drug history was taken at the time of the testing and neither of the group had any history of smoking, alcohol intake, diabetes , hypertension or arthritis at the time of enrolment to avoid bias.

Both AMD cases and Healthy unaffected individuals underwent testing for hsCRP and homocysteine.

At the time of blood collection, none of the individuals had a fluorescein angiogram, which can interfere,[15] with homocysteine testing.

A high-sensitivity CRP assay was chosen over regular CRP, as hsCRP assay is capable of measuring CRP at a concentration of0.007 mg/l.[16]

Results

Subsequent analysis was based on the 30 AMD affected individualsand 30 healthy unaffected individuals who were adjusted for, Age, sex and environmental factors. Mean ages were 68.1 years (range 55–85) for affected individuals and 65 years (range, 50– 80) for unaffectedindividuals.

Of the ARM affected individuals, 80% had Dry ARM and 20% had Wet ARM.

Mean hsCRP levels for affected and unaffected individuals were 7.10and 2.10 mg/l, respectively (P value<0.01 ;CI 95%; Range 0.18-21.67).

Mean homocysteine levels for affected and unaffected individuals were 15.89 and 9.67 mol/l, respectively (P value < 0.01; CI 95% ; Range 5.46-25.51).

Of the AMD affected individuals, 79.9% had Dry AMD and 20.1 % had Wet AMD.

Pearsons coorelation analysis was done between Homocysteine and hsCRP values of cases and controls and a statistic positive coorelation was found (p(ro)=0.582; P-value= 0.0001)

However in cases, Statistically only a slight positive coorelation was found between homocysteine and hsCRP (p=0.2 and P value = 0.05).

In our study no statistically significant coorelation was found between advanced age and Homocysteine levels of cases (p(ro)=0.05; P-value=0.78), neither did we find any statistiacally significant coorelation between advanced age and hsCRP (p=0.153; P value=0.477)

There was no statistical correletion between Homocysteine in Dry AMD versus Wet AMD.(Table-1), However hsCRP was raised in Wet AMD when compared to Dry AMD.

The data collected was interperated using Independent Samples Mann-Whitney U-Test using SPSS 20.0 software statisticalsoftware (IBM SPSS Statistics) (Table-1)

Table -1

		HCY Cases	HCY Controls	HsCRP Cases	HsCRP Controls	HCY Dry ARM	HCY Wet ARM
N	Valid	30	30	30	30	18	12
	Missing	0	0	0	0	12	18
Mean		15.8987	9.6753	7.1073	2.1023	16.2900	15.3133
Median		15.8250	9.7650	7.0700	1.4350	15.8250	15.4950
Mode		20.23	11.45	7.62	.18[a]	10.56[a]	20.23
Std. Deviation		3.76879	2.88951	3.34233	1.76383	4.01808	3.44478
Range		15.10	11.38	21.06	5.66	14.95	9.82
Minimum		10.41	5.46	.61	.18	10.56	10.41
Maximum		25.51	16.84	21.67	5.84	25.51	20.23
Sum		476.96	290.26	213.22	63.07	293.22	183.76

Discussion

Our study shows that levels of hsCRP and homocysteine are elevated significantly in individuals with AMD relative tosimilar unaffected controls, and these factors remain associated with AMD upon adjustment for covariates of potential influence.

Low-grade chronic inflammation is now recognized to play a pivotal role in the pathogenesis ofnumerous degenerative diseases, including atherosclerosis and Alzheimer's.[17] Our data support the role of chronic inflammation in the development of AMD.

Cellular remnants and debris from degenerate retinal pigment epithelial cells,sequestered between the retinal pigment epithelium basal lamina and Bruch's membrane, may constitute a chronic inflammatory stimulus.[18]High levels of monocyte activation,an early participant in the inflammatory cascade, correlatewith a 5-fold risk of developing neovascular AMD.[19]Aspirin therapy,[20] an antiinflammatory drug, and statin therapy,[20] which decreases CRP,[21]are associated with decreasedrates of choroidal neovascularization in patientswith AMD.

A small number of previous studies have produced dissimilar results regarding homocysteine and CRP in agerelatedmaculopathy (ARM). The National Health and Nutrition Examination Survey[22] found no association with homocysteine and AMD. Axer-Siegel et al[23]found thathomocysteine levels were significantly elevated in neovascular ARM relative to dry maculopathy or controls. Ourstudy found that homocysteine was significantly elevated inindividuals with AMD relative to controls. However there was no statistical difference in levels of Homocysteine in Dry AMD when compared to Wet AMD.

In the Cardiovascular Health Study,[24] there was no association between CRP and AMD, but this study also found noassociation with smoking, which has been found to be amajor risk factor in other studies.[5,12]In contrast, data fromthe Age-Related Eye Disease Study[25]showed that CRP wasan independent risk factor for AMD.

The present study shows that elevated hsCRP and homocysteine levels are associated with AMD, and supports therole of inflammation and

atherosclerosis in the pathogenesisof AMD. C-reactive protein potentially can be reduced[26] by physical activity, weight loss, and statintherapy. Studies assessing the effect of statin therapy, which reduces CRP, on AMD have had inconsistent results[20,,27-31] and have failed to show that statin therapy reduces or prevents AMD. Large-scale studies[32,33]evaluating homocysteine reduction with cardiovascular end points are underway. Further studies are needed to differentiate whichcommon disease mechanisms of chronic inflammation andatherosclerosis contribute to the pathogenesis of AMD

However, Since there is no cure for Age Related Macular Degeneration, prevention is the first approach to reduce vision loss. Detection and Control of modifiable risk factors markers (CRP and Homocysteine levels) could reduce the risk of developing Age Related Macular Degeneration, and may provide a rational for treatment modalities.

Commercial Interest: nil

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