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Association of blood levels of homocysteine, vitamin B12, and serum cholesterol with age related macular degeneration in the elderly population of India

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Running title: Blood levels of homocysteine, vitamin B12, and serum cholesterol with age related macular degeneration.

Abstract

Aim: To determine any association of age related macular degeneration (ARMD) with blood levels of homocysteine, vitamin B12, and serum cholesterol.

Methods: The levels of homocysteine, vitamin B12, and serum cholesterol in patients who were diagnosed with Age related macular degeneration (ARMD) in either eye (n=109) were compared to a control group with no retinal pathology (n=134). Diabetic patients were excluded from the study.

Results: Raised serum cholesterol levels were more common in ARMD cases (47.5%) compared to controls (28.4%). This association was found to be statistically significant ($p<0.01$). Levels of serum homocysteine were higher in the ARMD group than in the control group whereas serum vitamin B12 levels were lower in the ARMD group than in the control group but was not statistically significant ($p=0.68$ and $p=0.51$). Serum homocysteine level was significantly higher in males compared to females in both case and controls ($p<0.01$).

Conclusion: Raised serum cholesterol levels were significantly associated with increased risk of age related macular degeneration. Our study did not find elevated serum homocysteine or low serum vitamin B12 as significantly associated with age related macular degeneration.

Keywords: age-related macular degeneration, homocysteine, serum vitamin B12, serum cholesterol

Introduction

Age-related macular degeneration (ARMD) is an acquired degeneration of the retina that causes significant impairment of central vision. It is the leading cause of blindness in the developed world in people over 50 years. It is of two types: dry (atrophic) and wet (neovascular or exudative). This condition usually starts as dry type and in 10-20% of individuals, it progresses to the wet type. Dry ARMD includes drusen, abnormalities of RPE, hyperpigmentation and atrophy. Choroidal neovascular membrane (CNV) is the distinguishing feature of the wet form of ARMD[1].

The dry form is more frequently seen and leads to progressive loss of central vision. There is currently no treatment for dry age-related macular degeneration. Only nutritional support in the form of antioxidants can be given in early cases. The neovascular form of ARMD is more serious and can lead to profound loss of central vision. The treatment available is in the form of anti-vascular endothelial growth factor (VEGF) therapy that can only slow vision loss which is expensive and requires multiple injections[1]. ARMD can also be classified into early, intermediate, and late ARMD according to drusen size and pigmentary abnormalities present within 2 disc diameters of the center of the macula[2] (Table1).

ARMD is a multifactorial disease with etiopathogenesis

still not completely understood. The main risk factors are age, race, smoking, genetics, hypertension, high cholesterol and high body mass index[3]. There is some evidence that oxidative stress and antioxidants play a role in progression of ARMD[4]. Homocysteine is an intermediate formed during the metabolism of methionine, an essential sulphur-containing amino acid supplied from dietary proteins. Vitamin B12 as methylcobalamin is required for remethylation of homocysteine to methionine as coenzyme for methionine synthase. Blood levels of vitamin B12 are inversely correlated with blood homocysteine levels[5].

Epidemiological studies have shown that cases with coronary, cerebral and peripheral vascular disease have higher homocysteine levels than controls[6]. Elevated homocysteine promotes atherosclerosis through increased oxidant stress, impaired endothelial function, and induction of thrombosis. Hypercholesterolemia and elevated homocysteine level may raise the production of reactive oxygen species that can initiate lipid peroxidation and contributing to cardiovascular disease[7,8].

Thus, elevated homocysteine levels can be an important risk factor in the pathogenesis of ARMD by inducing oxidative stress and vascular dysfunction.

The causes of hyperhomocysteinemia are multifactorial. Elevated homocysteine levels are seen in nutritional deficiencies such as folate, vitamin B6, vitamin B12; increasing age, male sex and renal impairment[9]. Treatment of hyperhomocysteinemia is through vitamin supplementation of folic acid, vitamins B6 and B12[9].

The aim of our study is to compare levels of homocysteine, vitamin B12 and serum cholesterol between ARMD patients and subjects with no retinal pathology.

Materials and methods

This case-control study was conducted at the Ophthalmology department of tertiary hospital in district Hamirpur, Himachal Pradesh, India during April 2019 to April 2020. 109 patients above 50 years with ARMD were diagnosed and classified according to the severity stage as given in Table 1 based on fundus photography as cases group. The control group included 134 subjects selected from patients who came in for consultation in the Ophthalmology Department, without evidence of ARMD and without history of diabetes. They were matched with the cases in terms of age and sex. The study protocol was approved by the institutional ethics committee and written consent was obtained from the participants.

Inclusion criteria-

Patients were more than 50 years of age

Diagnosed with Early/ Intermediate or late ARMD in either eye (Table1)

Media sufficiently clear to take fundus photograph

Table 1: Classification of Age related macular degeneration based on fundus photograph

Characteristics	
Early ARMD	Medium-sized drusen>63 µm and ≤125 µm No pigmentary abnormalities
Intermediate ARMD	Any large drusen>125 µm or with pigmentary abnormalities associated with atleast medium drusen
Late ARMD	Neovascular AMD and/or any geographic Atrophy

Exclusion criteria:

Patients with sufficient media opacity to obscure fundus details and diabetic patients.

Patients' work up included detailed medical and systemic history including vitamin usage, assessment of visual acuity, slit lamp biomicroscopy for cataract grade, fundus examination and refraction. Fundus photography using a 45° digital nonmydriatic camera (Topcon) was performed through a pharmacologically dilated pupil. Two colour photographs were taken of each eye; the first centered on the optic disc and the second centered on the fovea. Fundus photographs were classified into Early ARMD (early or intermediate- photo 1 and photo 2) or Late ARMD (late /advanced ARMD- photo 3) based on classification given in table 1. Participants were classified according to the status of the worse eye as defined by disease severity.

A sample of 5 ml of venous blood was collected from all subjects after 12 hours fasting and was investigated for fasting blood sugar levels, serum homocysteine, vitamin B12 levels and serum cholesterol levels. Cut-off values for high total serum cholesterol were >200mg/dl, high HDL ≥

60mg/dl, low serum B12 <197 pg/ml and high serum Homocysteine> 13.56 micromol/L. Patients with fasting blood sugar levels > 100mg/dl or known diabetics were excluded from the study.

Serum Vitamin B12 were measured using competitive ECLIA method and serum Homocysteine using electrochemiluminescence method. Fasting blood sugar levels were measured using hexokinase method. Total cholesterol was measured using enzymatic method and HDL cholesterol was measured by direct measure (Immunoinhibition).

Data was analyzed and statistically evaluated using SPSS-PC-19 version.

Quantitative data was expressed in mean±standard deviation or median with interquartile range and difference between two comparable groups were tested by student's t-test (unpaired) or Mann Whitney 'U' test while qualitative data were expressed in percentage. Statistical differences between the proportions were tested by chi square test or Fisher's exact test. Odds ratio was calculated to see the risk in cases compared to controls. 'P' value less than 0.05 was considered statistically significant.

Results

The levels of homocysteine, vitamin B12, and total serum cholesterol in patients who were diagnosed with ARMD (n=109) were compared to a control group with no retinal pathology (n=134). ARMD patients were further classified into Early (n=86) and Late ARMD (n=23) (Fig.1, Fig. 2, Fig.3).

1. Mean age of ARMD cases was 69.87±10.52 years while mean age of controls were 67.10±8.15 years.
2. Mean age of males with ARMD was 70.61±10.97 years while mean age of females with ARMD was 68.96±9.98 years. There was no significant difference (p=0.41).

   In ARMD cases 55.0% were males while in controls 68.7% were males.

   In Late ARMD cases, 65.2% were males while in Early ARMD cases 52.3% were males.
3. History of smoking was present in 34.9% cases and 40.3% controls while hypertension was present in 4 (3.7%) ARMD cases and 24 (17.9%) controls. There was no history of vitamin usage in cases or controls.
4. The mean levels of serum vitamin B12 and serum homocysteine levels in ARMD cases were 409.96±409.92 pg/ml and 20.60±11.27 micromol/L respectively while the mean levels of serum vitamin B12 and serum homocysteine levels in controls were 381.88±374.01 pg/ml and 19.79±10.53 micromol/L respectively (Table 2) . No significant difference was seen (p=0.68 and p=0.51).

Table 2: Comparison of S. Vitamin B12, S. Homocysteine and age between cases and controls

	Group		p value
	Cases (n=109)	**Controls (n=134)**	
Age in years	69.87±10.52	67.10±8.12	0.04
S. Vitamin B12	409.96±409.92	381.88±374.01	
Median (IQR)	250 (167.45-461.40)	255.70 (192-419.8)	0.68
S. Homocysteine	20.60±11.27	19.79±10.53	
Median (IQR)	17.93 (13.17-24.35)	17.80 (11.95-24.54)	0.51

Low serum vitamin B12 was present in 36.7% in cases while in controls it was 26.9%.

Low serum Vitamin B_{12} was non-significantly associated with increased odds of ARMD (OR1.58, 95% CI 0.91 to 2.72).

The prevalence of increased serum homocysteine in cases was 72.5% while in controls it was 70.1%. Increased serum homocysteine was non-significantly associated with increased odds of ARMD (OR1.12, 95% CI 0.64 to1.96).

5. Serum Homocysteine levels in males in ARMD group and control group were 23.35±11.39 micromol/L and 22.31±10.22 micromol/L respectively while in females in ARMD group and control group was 17.22±10.27 micromol/L and 14.25±9.17 micromol/L respectively.

   Mean serum homocysteine level was significantly higher in males compared to females in both case and controls (p<0.01). There was no significant difference in mean serum vitamin B12 level between males and females (p=0.14).

6. Low serum vitamin B12 levels was observed in 47.8% cases of Late ARMD and 33.7% cases of Early ARMD. There was no statistically significant difference (p=0.21) Table 3.

Table 3: S. Vitamin B_{12} in Late and Early ARMD study subjects

S. Vitamin B_{12}	Late ARMD (n=23)		Early ARMD (n=86)		P value
	No.	%	No.	%	
Low	11	47.8	29	33.7	
Normal	12	52.2	57	66.3	0.21

S. Homocysteine was high in 82.6% cases of Late ARMD and 69.8% cases in Early ARMD. No significant difference was observed between the 2 groups. (p= 0.29) Table 4.

Table 4: S. Homocysteine in late and early ARMD study subjects

S. Homocysteine	Late ARMD (n=23)		Early ARMD (n=86)		P value
	No.	%	No.	%	
Normal	4	17.4	26	30.2	
High	19	82.6	60	69.8	0.29

7. Raised serum cholesterol was more common in ARMD cases (47.5%) compared to controls (28.4%) (Table 5). This association was found to be statistically significant (p<0.01).

Raised serum cholesterol levels were more commonly seen in Early ARMD cases (51.2%) compared to Late ARMD cases (34.8%) but this association was not found to be statistically significant (p=0.16).

Table 5: S. Cholesterol in study subjects

S. Cholesterol	Cases (n=109)		Controls (n=134)		P value
	No.	%	No.	%	
Normal	57	52.3	96	71.6	
High	52	47.7	38	28.4	<0.01

Fig:1 Fundus Photo 1: Intermediate ARMD

Fig:2 Fundus Photo 2: Early ARMD

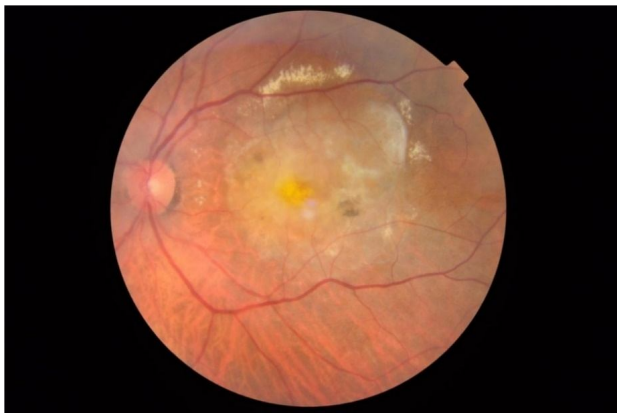

Fig:3 Fundus Photo 3: Late /Advanced ARMD

Discussion

Age related macular degeneration is a chronic, progressive degenerative disorder of the macula and features central visual loss as a result of drusen deposition, geographical atrophy, serous detachment of the retinal pigment epithelium and neovascularization. It is one of the leading causes of irreversible visual impairment among the elderly worldwide. The cause of ARMD is multifactorial, influenced by various genetic and enviromental factors such as age, ethnic background, dietary factors, lifestyle factors etc. Atherosclerosis and oxidative damage has also been implicated in the aetiopathogenesis of ARMD. Hypercholesterolemia and hyperhomocysteinemia may increase oxidative stress and hence promote endothelial dysfunction[1].

ARMD is not curable. Vitamin supplementation, dietary modification, and cessation of smoking may slow the progression of vision loss in dry ARMD. Dietary levels of folate and vitamin B12 are inversely related to plasma homocysteine levels. Thus, folic acid and vitamin B12 supplementation can decrease serum homocysteine levels. Identifying modifiable risk factors such as nutritional deficiencies and altered lipid profile in the aetiopathogenesis of ARMD and correcting them may prevent substantial vision loss[11].

Our study aimed to assess the role of biochemical parameters such as homocysteine, vitamin B12 levels and cholesterol levels in patients with ARMD.

In our study we did not find any male gender predominance in early or advanced ARMD cases. However, in a hospital based study in patients more than 50 years of age in Maharashtra they found male predominance in ARMD patients[10].

In our study we did not find any significant association of smoking and hypertension in ARMD cases compared to controls. Although previous studies have shown smoking to be a modifiable risk factor in the progression of ARMD. According to ARED study report number 3, the avoidance of smoking and the prevention of hypertension, may reduce the risk of developing ARMD[11]. In a study done in ARMD group (Vine AK et al) older age and higher homocysteine were risk factors for ARMD while no significant differences was found between cases and controls in terms of gender, hypertension and smoking[12].

In our study we did not find any significant association of serum vitamin B12 and serum homocysteine levels with early or late ARMD.

A study done comparing homocysteine levels between neovascular or wet ARMD and dry ARMD (Axer-Siegel R et al and Ghosh S et al) found hyperhomocysteinemia to be significantly associated with the wet ARMD variety but not with the dry ARMD[13,14]. In another study (Gopinath B et al) elevated serum homocysteine and folate and vitamin B-12 deficiencies predicted increased risk of incident ARMD and suggested a potential role for vitamin B-12 and folate in reducing ARMD risk[15].

In another study (Pinna A et al) in early and neovascular Age-Related Macular Degeneration found a very weak association of ARMD with increased plasma homocysteine[16]. In a study done (Christen W. G et al) in a large cohort of apparently- healthy women and men, they did not find a strong role for homocysteine in ARMD occurrence[17,18].

In our study we found serum homocysteine to be significantly raised in males compared to females in both cases and controls.

In our study, serum cholesterol levels were found to be significantly elevated in both dry and wet type of ARMD cases compared to the control group. Previous studies have found raised cholesterol levels to be associated with ARMD cases. In a case – control study (Davari MH et al) in elderly patients it was found that high level of total cholesterol was associated with increased risk of ARMD[19].

Conclusion

We found raised levels of total serum cholesterol to be associated with Age related macular degeneration.

In our study, we did not find significant association between hyperhomocysteinemia or low serum vitamin B12 in patients with age related macular degeneration, hence routine testing of these is not proposed.

Limitation

Our study was based on evaluation of single sample of homocysteine, serum vitamin B12 and cholesterol level. We

did not take consideration of the fact that changes of these biochemical parameters can occur over time due to several factors that can influence the study. Further large prospective studies are needed to determine any possible association between serum vitamin B12 and serum homocysteine and the risk of ARMD.

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Ethics declarations

Conflict of interest

Anushree Gupta, Anil Chauhan, all authors have no conflicts of interest that are directly relevant to the content of this review.

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