Dietary and lifestyle risk factors associated with age-related macular degeneration: A hospital based study

Bhatiwada Nidhi, Bangera Sheshappa Mamatha, Chamrajnagar Anantharajiah Padmaprabhu[1], Prabhu Pallavi[1], Baskaran Vallikannan

Aim: To establish the frequency, associations and risk factors for age-related macular degeneration (AMD) in hospital population of South India. **Materials and Methods**: In this cross-sectional hospital based study, 3549 subjects (2090 men and 1459 women) above 45 years of age were screened randomly for AMD. Participants underwent ocular evaluation and were interviewed for lifestyle variables and dietary intake of carotenoids by structured food frequency questionnaire. AMD was defined according to the international classifications and grading system. **Results**: Either form of AMD was detected in 77 (2.2%) participants. Of which, early and late AMD was present in 63 (1.8%) and 14 (0.4%) subjects, respectively. Binary logistic analysis showed that the incidence of AMD was significantly higher with increasing age (Odds ratio [OR] 1.17; 95% CI 1.13-1.22) and diabetes (OR 3.97; 95% CI 2.11-7.46). However, AMD was significant among heavy cigarette smokers (OR 5.58; 95% CI 0.88-7.51) and alcoholics (OR 4.85; 95% CI 2.45-12.22). Dietary lutein/zeaxanthin (L/Z) and β-carotene intake were associated ($P < 0.001$) with the reduction in risk for AMD, with an OR of 0.38 and 0.65, respectively. **Conclusions**: Higher dietary intake of carotenoids, especially L/Z, was associated with lower risk for AMD. Risk of AMD is higher with increasing age and was prevalent among subjects with diabetes. Cessation of smoking and alcohol may reduce the risk of AMD in this population.

Key words: Age-related macular degeneration, carotenoids, cross-sectional studies, lutein

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in elderly populace and accounts for 8.7% of all cases of blindness globally.[1] Estimates indicate that eight million people will be affected with AMD worldwide by the year 2020; from that 10 to 20% will be late AMD responsible for approximately 90% of vision loss.[2] Although, there is considerable information on visual impairment in the Western world, to date there are only few studies on the prevalence and risk factors of AMD in Indian subcontinent.[3] Recent studies from India account the prevalence of AMD among 70 years and above as 2% and 3.7% which was comparable to Western countries.[3,4] Therefore, the investigation of risk factors of AMD is important in comprehending the disease and to suggest preventive measures that can retard or control AMD progression. Risk factors, such as hypertension, smoking, diabetes mellitus, cardiovascular disease, obesity, female sex and positive family history.[4-9] However, to some extent, the progression of AMD can be slow downed through consumption of lutein/zeaxanthin (L/Z) rich food in the diet.[10-12] Hence, the purpose of the present study was to describe the prevalence of AMD along with an investigation of putative risk factors-principally environment, lifestyle and diet in a hospital population of Southern India.

Department of Molecular Nutrition, CSIR-Central Food Technological Research Institute, CSIR, Mysore, [1]Department of Ophthalmology, Sushrutha Eye Hospital, Mysore, Karnataka, India

Corresponding Author: Dr. V. Baskaran, Department of Molecular Nutrition, CSIR-Central Food Technological Research Institute, Mysore - 570 020, Karnataka, India. E-mail: baskaranv@cftri.res.in

Materials and Methods

In this cross sectional hospital based descriptive study; consecutive patients aged 45 years and above visiting tertiary eye care centre were screened for AMD between June and December 2009. However, patients with follow up visit and any ocular surgery were excluded from the study. Before screening and definitive examination, the study was explained in detail to all the participants. Verbal or written consent was obtained from all the participants in accordance with the World Medical Association's Declaration of Helsinki. The study population included 4378 new patients visited the eye hospital, of whom 3549 subjects (81%) participated in the study, were examined in the clinic between 2010 and 2011. The remaining 829 (19%) were classified as non-participants.

An interviewer administered questionnaire was used to collect information on socio-demographic, personal medical history and lifestyle factors. Self reported hypertension or diabetes and its duration from diagnosis were recorded. Height and weight of all subjects were measured and documented to find out the body mass index (BMI).

All the ophthalmic examination was performed by experienced ophthalmologists. The examination was conducted according to a standardized protocol that included visual acuity, autokeratometer refractometer (KR-8100; Topcon, Tokyo, Japan), computerized tonometer (CT-80; Topcon, Tokyo, Japan) and slit lamp biomicroscopy (SL-IE; Topcon, Tokyo, Japan) with 90 D and 78 D lens through a dilated pupil with tropicamide (0.8%) and phenylephrine (5%). For grading lens opacity, each eye was compared with the Lens Opacities Classification System (LOCS III) photographs.[13] A modified classification of diabetic retinopathy was used in our study.[14]

Anterior segment was photographed with a camera (Nikon Corporation, Tokyo, Japan) mounted to slit lamp with a fundus camera (Kowa VX-10, Japan). Photographs were classified according to an international classification and grading system of age related macular degeneration.[15] Fundus photograph were characterized to know the type of lesion/drusen [Fig. 1]. The features were examined for hard and soft drusens, changes in the retinal pigment epithelium (RPE), geographic atrophy, choroidal neovascular membrane, and disciform scar. AMD was classified as late (neovascular or geographic atrophy) or early (soft drusen or retinal RPE abnormalities), they were combined for analysis in the present study. The cases of AMD, thus, detected were also confirmed by the principal ophthalmologist. When both the eyes of participants had lesions of different severity, the grade assigned for the participant was that of the more severely involved eye.

Daily dietary intake of carotenoids (L/Z and β-carotene) was estimated by a structured food frequency questionnaire. Since, the frequency of one measure taken at a single time point does not represents subsequent intake over time, we used a structured food frequency questionnaire for better representation of the habitual dietary intake for our study. Questionnaire included seasonal items, their amounts per serving and number of serving per day, as well as frequency of the item per day/week/month, in order to obtain a representative picture of the dietary practice over a long period of time.

All the commonly consumed food having carotenoids were categorised into seven food groups like cereals, legumes, vegetables, green leafy vegetable, fruits, dairy products and animal foods. Standard measures such as number, cup, glass, plate, bowl, teaspoon and tablespoon were used to measure the consumption of each item as weight in grams per day. Intake of L/Z and β-carotene was calculated based on the database generated by extensive screening of food samples.[16-18]

Statistical analysis

The association of AMD with risk factors noted was assessed by bivariate analysis, using Chi Square Test and Fisher's Exact Test (only when the expected frequencies were less than 5). Variables associated with AMD ($P < 0.25$) in bivariate analysis were further tested in binary logistic regression model adjusting for potential confounders and interactions. The binary logistic regression analysis was used to identify risk factors that placed an individual at risk of AMD (early and late combined) in one or both eyes. Odd ratios (ORs) and 95% confidence interval (CI) were calculated using logistic regression with variance calculation by statistical software package (IBM SPSS Statistics 20, Chicago, USA).

Results

A total of 4378 new subjects visited the eye hospital above 45 years and older were enumerated and of those, 3549 subjects (81% response rate) participated in the study. The study population was representative of both the urban and rural population of Karnataka, India. For urban residents, the age ranged from 45 to 87 years (59.76 ± 8.25; median, 60 years), and 1567 (59.3%) were men. The age of rural residents ranged between 45 and 86 years (59.5 ± 8.45; median, 50 years); 523 (57.6%) were men [Table 1].

Either form of AMD (early and late) was detected in 77 (2.2%) of 3549 participants aged ≥ 45 years with a median age of 69 (68.55 ± 9.44) years. Early AMD was present in 63 (1.8%) participants and late AMD was present in 14 (0.4%) subjects [Table 1]. Of the 77 subjects with AMD, nine (11.7%) were blind or had only hand movement vision in the affected eyes [Table 1]. The prevalence of any, early and late AMD increased from 0.9%,0.7%,0.2%, respectively, in the 50-54 year age group, to 1.8%,1.6%, .3%, respectively, in the 65-69 years old group and to 12.7%,9.4%,3.3%, respectively, in the group aged 75 years and older [Table 1]. Table 2 and 3 reports the distribution of AMD. Neither place of living, eating habits, family size, socio- economic status, physical activity nor education was associated with AMD [Table 2]. The potential risk factors found significant in the bivariate analysis were age ($P < 0.0001$), diabetes ($P = 0.003$), diabetic retinopathy ($P = 0.002$), BMI ($P < 0.0001$), smoking ($P= 0.017$) and alcohol consumption ($P = 0.001$) [Table 3].

Figure 1: Representative fundus photograph of early and late AMD

Table 1: Prevalence of age-related macular degeneration in participants stratified by age, gender and place of living

Characteristics		Any AMD	Early AMD	Late AMD
Age groups (years)	No. at Risk (%)	*n* (%)	*n* (%)	*n* (%)
45-49	378 (10.6)	2 (0.5)	2 (0.5)	0 (0)
50-54	562 (15.8)	5 (0.9)	4 (0.7)	1 (0.2)
55-59	846 (23.8)	5 (0.6)	3 (0.4)	2 (0.2)
60-64	652 (18.4)	15 (2.3)	15 (2.3)	0 (0)
65-69	707 (19.9)	13 (1.8)	11 (1.6)	2 (0.3)
70-74	224 (6.3)	14 (6.3)	11 (4.9)	3 (1.3)
75+	180 (5.1)	23 (12.7)	17 (9.4)	6 (3.3)
Total population	3549	77 (2.2)	63 (1.8)	14 (0.4)
Men	2090 (58.9)	40 (1.9)	30 (1.4)	10 (0.5)
Women	1459 (41.1)	37 (2.5)	33 (2.3)	4 (0.3)
Rural	908 (25.5)	21 (2.3)	15 (1.7)	6 (0.7)
Urban	2641 (74.4)	56 (2.1)	48 (1.8)	8 (0.3)

n refers to cases and values given in the parenthesis refers prevalence of age related macular degeneration in population at risk. AMD: Age-related macular degeneration
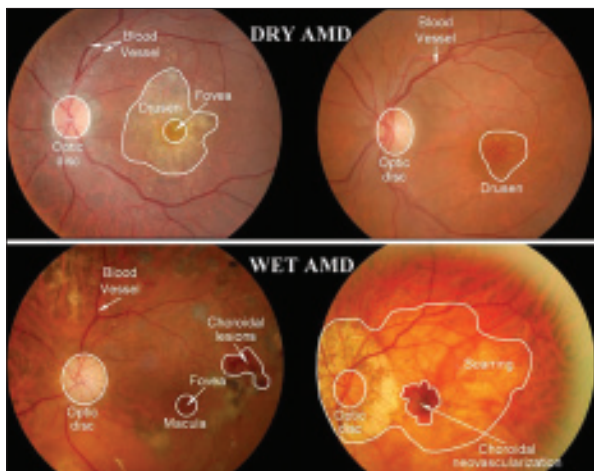
The mean daily intake of L/Z and β-carotene varies from 0.6 to 5.98 and 0.4 to 4.62 mg per day, while the mean dietary intake of these carotenoids were 1.97 ± 1.09 mg/day and 1.54 ± 0.94 mg/day, respectively [Table 4]. Higher levels of carotenoid intake were associated with a reduced risk for AMD. There was a statistically significant, apparently linear trend for a reduction of AMD risk with increasing amount of carotenoids in the diet. When individual carotenoid were evaluated for risk of AMD, higher dietary intakes β-carotene and L/Z were associated ($p < 0.001$) with reduced risk of AMD, with an OR of 0.38 and 0.65, respectively [Table 5].

In a binary logistic regression that adjusted for potential confounders and for interactions between age and AMD, increasing age (OR = 1.17; 95% CI 1.13-1.22) was significantly associated with AMD [Table 5]. Incidence of AMD was higher in women (OR = 1.43; 95% CI 0.70 to 2.99) than in men in the study population. The presence of AMD was significantly higher among participants having diabetes (OR = 3.97; 95% CI 2.1 1 to 7.46). However, prevalence of AMD was marginally significant among

moderate smokers (OR = 2.97; 95% CI 1.03-8.65) and heavy smokers (OR = 5.58; 95% CI 0.88 to 7.51). The odds of the presence of AMD among moderate and heavy cigarette smokers were higher than the never smoked reference group. Likewise, incidence of AMD was significantly higher in light (OR = 3.35; 95% CI 1.21 to 9.21) and heavy (OR = 4.85; 95% CI 2.45 to 12.22) drinkers [Table 5].

Discussion

Data from this hospital based study demonstrated an expected association between age and AMD. Cigarette smoking, alcohol

Table 2: Associations between AMD and demographic factors in the study population

Characteristics	Total population (N=3459)	AMD n (%)	P value
Gender			
Male	2090	40 (1.9)	0.211*
Female	1459	37 (2.5)	
Place of living			
Urban	2641	56 (2.1)	0.731*
Rural	908	21 (2.3)	
Eating habit			
Vegetarian	1791	46 (2.6)	0.100*
Non vegetarian	1758	31 (1.8)	
Education			
Illiterate	2102	54 (2.6)	0.136*
Literate	1447	23 (1.6)	
Socioeconomic status‡			
Extreme lower	865	20 (2.3)	0.629*
Lower	1209	30 (2.5)	
Middle	958	19 (2)	
Upper	517	8 (1.5)	
Family size§			
Small	1919	41 (2.1)	0.272*
Medium	1481	34 (2.3)	
Large	149	2 (1.3)	
Physical activity			
Sedentary	3358	76 (2.3)	0.412†
Moderate	176	1 (0.6)	
Heavy	15	0 (0)	

*χ^2 test, †Fisher exact test ‡Socioeconomic status defines according to monthly income in Indian rupees: ≤1000: Extreme lower, 1001-5000, lower; 5001-10000: Middle, ≥10001 upper. §Family size defines number of person in the family: 1-4: small, 5-8: Medium, ≥9: Large, AMD: Age-related macular degeneration

Table 3: Association between AMD and potential risk factors

Characteristics	Total population (N=3459)	AMD n (%)	P value
Hypertension			
No	2093	40 (1.9)	0.205*
Yes	1456	37 (2.5)	
Diabetes			
No	2016	31 (1.5)	0.003*
Yes	1533	46 (3)	
Cataract			
No	2549	55 (2.1)	0.938*
Yes	1000	22 (2.2)	
Diabetic retinopathy			
No	2770	71 (2.6)	0.002*
Yes	779	6 (0.8)	
Body mass index			
Under weight (BMI<20)	729	30 (4.1)	<0.0001*
Desirable weight (BMI 20-25)	1759	35 (2)	
Over weight (BMI 25-30)	867	9 (1)	
Obese (BMI>30)	194	3 (1.5)	
Tobacco†			
Never consumed	3219	68 (2.1)	0.752*
Moderate	287	8 (2.8)	
Heavy	43	1 (2.3)	
Smoking‡			
Never a smoker	3305	66 (2)	0.017*
Moderate smoker	200	8 (4)	
Heavy smoker	44	3 (6.8)	
Alcohol consumption§			
Never a drinker	3279	63 (1.9)	0.001*
Light drinker	213	10 (4.7)	
Heavy drinker	57	4 (7)	

*χ^2 test, †A person who consumes tobacco for 1 to a day was considered to be moderate and if consume ≥3 times was considered as heavy. ‡A person who smokes 1 to 5 cigarettes in a day was considered to be moderate smoker and if a person smokes ≥6 cigarettes in a day was considered as heavy smoker. §A person who drinks alcohol for 1 to 2 times a week was considered to be light drinker and if drinks 3 to 4 times a week or 5 to 6 times a week was considered as heavy drinker, AMD: Age-related macular degeneration

Table 4: Association between AMD and dietary intake of carotenoids

Carotenoid intake	Total population (*N*=3459)	AMD *n* (%)	*P* value
L/Z intake (mg/day)			
0-1	961	35 (3.6)	0.001*
1-2	950	25 (2.6)	
2-3	1117	17 (1.5)	
3-4	480	0	
4-5	37	0	
5-6	4	0	
β-carotene intake (mg/day)			
0-1	1322	45 (3.4)	0.001*
1-2	1341	21 (1.5)	
2-3	639	11 (1.7)	
3-4	224	0	
4-5	23	0	

*χ^2 test, AMD: Age-related macular degeneration

Table 5: Bivariate analysis on the association between AMD with ocular and general parameters

Characteristics	OR (95% CI)	*P* value
Age	1.17 (1.13-1.22)	0.000
Female	1.45 (0.70-2.99)	0.32
Urban	0.99 (0.50-1.95)	0.97
Non vegetarian	0.77 (0.42-1.41)	0.39
Hypertension	1.22 (0.67-2.23)	0.51
Diabetes	3.97 (2.11-7.46)	0.016
Non proliferative diabetic retinopathy	0.021 (0.007-0.066)	0.094
Proliferative diabetic retinopathy	0.035 (0.003-0.38)	0.116
Coronary heart disease	1.83 (0.61-5.53)	0.280
Tobacco*		
Moderate	0.66 (0.22-1.94)	0.449
Heavy	0.64 (0.02-1.78)	0.794
Smoking†		
Moderate smoker	2.97 (1.03-8.65)	0.045
Heavy smoker	5.58 (0.88-7.51)	0.068
Alcohol consumption‡		
Light drinker	3.35 (1.21-9.21)	0.019
Heavy drinker	4.85 (0.45-8.22)	0.023
L/Z intake	0.38 (0.33-0.69)	0.000
β-carotene intake	0.65 (0.42-0.86)	0.000

*A person who consumes tobacco for 1 to a day was considered to be moderate and if consume ≥ 3 times was considered as heavy. †A person who smokes 1 to 5 cigarettes in a day was considered to be moderate smoker and if a person smokes ≥ 6 cigarettes in a day was considered as heavy smoker. ‡A person who drinks alcohol for 1 to 2 times a week was considered to be light drinker and if drinks 3 to 4 times a week or 5 to 6 times a week was considered as heavy drinker

consumption and diabetes were also significantly associated with AMD. An inverse trend associated with carotenoid intake (lower risk for AMD with higher intake) was evident in the study population.

Since, the study sample was randomly selected from all the cases at the time of their first registration at hospital; it represents the prevalence rate of AMD of those registered. Although the registration and screening were done at different times the data should be considered as point prevalence. Our estimate of AMD (2.2%) from the hospital was similar to that reported in Indian population as 1.82% in the Andhra Pradesh Eye Disease Study[4] and Western population as 1.51% in the Beaver Dam Eye Study[19] and 1.81% in the Blue Mountain Eye Study.[20] These slight differences in the incidence of AMD among the studies could be due to the differences in environmental exposure among the population, genetic factors or perhaps to the difference in the methodology adopted. Present study reports 1.8% of early AMD in the subjects visited hospital, which was similar to that accounted in Chinese population as 1.4% (The Beijing Eye Study),[21] but it was lower than the study from South Indian population as 2.7% (Aravind Comprehensive Eye Study) and Singapore Malay Eye Study (4.9%).[3,22] However, prevalence of late AMD in the present study population was 0.4%, which was in line with Aravind Comprehensive Eye Study (0.6%),[3] Beijing Eye Study (0.2%),[21] the Singapore Malay Eye Study (0.7%),[22] but it was lower than the Blue Mountain Eye Study (1.1%).[20] The prevalence of AMD among 75 +years found in this study was 12.7% as similar to MESA whites (13.3%),[23] but was much lower than in the Beavers Dam whites (36.8%).[19] Results clearly demonstrated that prevalence of AMD in 75 + years is approximately 4.4 fold higher than that of 65 to 74 years. Therefore, increasing age is strongly associated with AMD.[3,4,19-23]

The present study, as well as previous studies, found that the incidence of early AMD significantly increased with advancing age in women and the incidence of late AMD significantly increased with advancing age in men.[19,20] However, we found no such correlation between age and late AMD in women. This difference may have resulted due to low incidence of late AMD among women. Gender differences in AMD prevalence have been inconsistently reported, with a higher prevalence of late AMD in men.[23] In contrast, Western white population, have shown a higher prevalence of AMD in women.[5,7,9]

Cigarette smoking was constantly been identified as a risk factor for AMD.[4-8] Present study also showed a strong association between heavy smoking (OR 5.58) and AMD. Alcohol intake as a risk for AMD have shown inconsistent findings.[24,25] Alcohol consumption (OR 3.35) and risk of AMD showed a strong alliance, which was similar to Los Angles Latino Eye Study.[25] There are conflicting reports relates to association of hypertension and AMD. We did not find hypertension to be associated with AMD in the sample population.[3] However; evidenced higher odds (1.22) of AMD in the hypertensive group. On the other hand, diabetes (OR 3.97) showed strong association with AMD, which was in line with European Eye Study (OR 1.81).[26]

The current study showed relationship between BMI and AMD, which is consistent with Blue Mountain Eye Study.[20] In contrast a number of other studies have recorded no relationship between BMI and AMD.[4,24] Presence and amount of any cataract were not significantly associated with an increased risk of AMD.[21] It is in contrast with previous studies

in which a higher prevalence of AMD was reported in the presence of cortical cataract or cataract surgery.[4,7,8] Consistent with the existing literature, the present study did not find any association between AMD and education or socio-economic status or place of living or eating habits.[4,24]

Mean dietary intake of L/Z was 1.97 ± 1.09 mg/day, these values are comparable with those obtained by previous investigators in similar age groups.[12] Finding from this study supports the possibility that an increased intake of dietary carotenoids may reduce the risk of AMD, associated with severe visual loss. Odd ratios obtained for L/Z and β-carotene (0.38 and 0.65) was comparable to a Western Multicenter Eye Disease Case-Control Study (0.43 and 0.59), respectively.[10] Of both the carotenoids evaluated, the combination of L/Z was most strongly associated with AMD. However, suggestive trend for an inverse association between β-carotene intake and AMD but this appeared to be entirely due to the carotenoid rich foods.[10] These findings regarding dietary intake of carotenoids are consistent with previous report on dietary and serum L/Z, further strengthen and confirms the present findings.[11]

Limitation and potential biases of this hospital study should be noted. Fundus photographs (11.8%) were of insufficient quality for AMD grading, mainly due to media opacity (cornea or lens) among older population, could lead to underestimation of AMD incidence. Relatively few cases of AMD, reduces the power of the study to identify all risk factors. The strengths of this study are the representativeness of the sample population, the high response rate and the standardised protocol, including the photographic documentation of macula.

In conclusion, the results are consistent with the hypothesis that increasing dietary intake of foods rich in carotenoids may reduce the risk of developing AMD. A significant trend was seen for a decreased risk for AMD among subjects with greater intake of carotenoids, especially lutein/zeaxanthin. This study also indicates that prevalence of AMD increased with increasing age and was common in patient having diabetes. Results also confirmed that higher prevalence of AMD in those who drink alcohol and smoke cigarette heavily. Alteration in these modifiable risk factors assumes greater importance if viewed within the context of greater health benefits not necessarily limited to AMD. Most importantly, identifying dietary strategies to prevent or retard the onset of AMD will have a major impact on the burden of blindness and visual impairment among the elderly.

Acknowledgments

References

1. Wong TY, Loon SC, Saw SM. The epidemiology of age related eye diseases in Asia. Br J Ophthalmol 2006;90:506-11.

2. Bressler NM. Early detection and treatment of neovascular age related macular degeneration. J Am Board Fam Pract 2002;15:142-52.

3. Nirmalan PK, Katz J, Robin AL, Tielsch JM, Namperumalsamy P, Kim R, et al. Prevalence of vitreoretinal disorders in a rural population of Southern India: The Aravind Comprehensive Eye Study. Arch Ophthalmol 2004;122:581-6.

4. Krishnaiah S, Das T, Nirmalan PK, Nutheti R, Shamanna BR, Rao GN, et al. Prevalence of vitreoretinal disorders in a rural population of southern India: The Aravind Comprehensive Eye Study. Invest Ophthalmol Vis Sci 2005;46:4442-9.

5. Age-Related Eye Disease Study Research Group. Risk factors associated with age-related macular degeneration-A case-control study in the age-related eye disease study: Age-Related Eye Disease Study report number 3. Ophthalmology 2000;107:2224-32.

6. Cackett P, Wong TY, Aung T, Saw SM, Tay WT, Rochtchina E, et al. Smoking, cardiovascular risk factors and age-related macular degeneration in Asians: The Singapore Malay Eye Study. Am J Ophthalmol 2008;146:960-7.

7. Klein R, Klein BE, Wong TY, Tomany SC, Cruickshanks KJ. Association of cataract and cataract surgery with the long-term incidence of age-related maculopathy: The Beaver Dam Eye Study. Arch Ophthalmol 2002;120:1551-8.

8. Wang JJ, Klein R, Smith W, Klein BE, Tomany S, Mitchell P. Cataract surgery and the 5-year incidence of late stage age-related maculopathy: Pooled finding from the Beaver Dam and Blue Mountains eye studies. Ophthalmology 2003;110:1960-7.

9. Klein R, Peto T, Bird A, Vannewkirk MR. The epidemiology of age-related macular degeneration. Am J Ophthalmol 2004;137:486-95.

10. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, et al. Dietary carotenoids, vitamin A, C and E and advanced age related macular degeneration. Eye disease case control study group. J Am Med Assoc 1994;272:1413-20.

11. Gale CR, Hall NF, Phillips DI, Martyn CN. Lutein and zeaxanthin status and risk of age-related macular degeneration. Invest Ophthalmol Visual Sci 2003;44:2461-5.

12. Rock CL, Thornquist MD, Neuhouser ML, Kristal AR, Neumark-Sztainer D, Cooper DA, et al. Diet and lifestyle correlates of lutein in the blood and diet. J Nutr 2002;132:525S-30.

13. Chylack LT Jr, Wolfe JK, Singer DM, Leske MC, Bullimore MA, Bailey IL, et al. The Lens Opacities Classification System III. The longitudinal study of cataract study group. Arch Ophthalmol 1993;111:831-6.

14. Klein R, Klein BE, Magli YL, Brothers RJ, Meuer SM, Moss SE, et al. An alternative method of grading diabetic retinopathy. Ophthalmology 1986;3:1183-7.

15. Bird AC, Bressler NM, Bressler SB, Chisholm IH, Coscas G, Davis MD, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. Surv Ophthalmol 1995;39:367-74.

16. Lakshminarayana R, Raju M, Krishnakantha TP, Baskaran V. Determination of major carotenoids in few Indian leafy vegetables by high-performance liquid chromatography. J Agric Food Chem 2005;53:2838-42.

17. Raju M, Varakumar S, Lakshminarayana R, Krishnakantha TP, Baskaran V. Carotenoid composition and vitamin A activity of medicinally important green leafy vegetables. Food Chem 2007;101:1621-8.

18. Mamatha BS, Sangeetha RK, Baskaran V. Provitamin-A and xanthophyll carotenoids in vegetables and food grains of nutritional and medicinal importance. Int J Food Sci Tech 2011;46:315-23.

19. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy:

The Beaver Dam Eye Study. Ophthalmology 1992;99:933-43.

20. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia: The Blue Mountains Eye Study. Ophthalmology 1995;102:1450-60.

21. Xu L, Li Y, Zheng Y, Jonas JB. Associated factors for age related maculopathy in the adult population in China: The Beijing Eye Study. Br J Ophthalmol 2006;90:1087-90.

22. Kawasaki R, Wang JJ, Aung T, Tan DT, Mitchell P, Sandar M, *et al*. Prevalence of age-related macular degeneration in a Malay population: The Singapore Malay Eye Study. Ophthalmology 2008;115:1735-41.

23. Klein R, Klein BE, Knudtson MD, Wong TY, Cotch MF, Liu K, *et al*. Prevalence of age-related macular degeneration in 4 racial/ethnic groups in the multi-ethnic study of atherosclerosis. Ophthalmology 2006;113:373-80.

24. Klein R, Klein BE, Jensen SC, Mares-Perlman JA, Cruickshanks KJ, Palta M. Age-related maculopathy in a multiracial United States population. The National Health and Nutrition Examination Survey III. Ophthalmology 1999;106:1056-65.

25. Fraser-Bell S, Wu J, Klein R, Azen SP, Varma R; Eye Study Group. Smoking, alcohol intake, estrogen use and age-related macular degeneration in Latinos: The Los Angles Latino Eye Study. Am J Ophthalmol 2006;141:79-87.

26. Topouziz F, Anastasopoulos E, Augood C, Bentham GC, Chakravarthy U, de Jong PT, *et al*. Association of diabetes with age-related macular degeneration in the EUREYE study. Br J Ophthalmol 2009;93:1037-41.