Serum Homocysteine, Vitamin B12, and Folate, and the Prevalence and Incidence of Posterior Subcapsular Cataract

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Purpose. We assessed associations between serum levels of homocysteine, vitamin B12, and folate, and the prevalence and 5-year incidence of posterior subcapsular cataract (PSC) in Blue Mountains Eye Study participants.

Methods. We examined 3508 participants aged 49+ years during 1997 to 2000, including 2334 (75.1% of survivors) original and 1174 (85.2% of those eligible) newly recruited subjects. Five years later (2002–2004), 1952 (76.6% of survivors) original participants were re-examined. Detailed examinations, including lens photographs and fasting blood tests, were conducted at both visits. Logistic regression models estimated odds ratios (OR) and 95% confidence intervals (CI) after multivariable adjustment.

Results. In this population, those with PSC were older, less likely to have higher education, and more likely to have diabetes and myopia. The PSC prevalence was 5.7% (150/2644). Higher levels of homocysteine (per SD; OR, 1.17; 95% CI, 1.00–1.37) and lower levels of folate (per SD; OR, 1.24; 95% CI, 0.99–1.56) were associated with prevalent PSC. There was significant interaction ($P < 0.05$) between vitamin B12 and homocysteine; for B12 $\geq$125 pmol/L, 28% higher PSC prevalence was associated with homocysteine (per SD; OR, 1.28; 95% CI, 1.09–1.52); however, for B12 <125 pmol/L, nonsignificant lower PSC prevalence was associated with homocysteine (per SD; OR, 0.16; 95% CI, 0.02–1.57). The 5-year PSC incidence was 5.7% ($n = 59/1030$) with no significant associations with homocysteine, B12, and folate.

Conclusions. Higher serum homocysteine level was associated with PSC prevalence in this population. Vitamin B12 status seemed to modify this association. Lack of longitudinal association could have resulted from insufficient study power.

Keywords: cataract, epidemiology, homocysteine

Serum homocysteine levels have been linked to eye diseases, such as venous and arterial occlusions,[1] retinal emboli,[2] glaucoma,[3,4] and age-related macular degeneration.[5] However, the link between homocysteine and cataract formation has not been fully investigated.[6] Recently, it was hypothesized that homocysteine may be a lens epithelial stressor, resulting in the development of unfolded protein aggregates in the lens.[7,8] In human and animal lens epithelial cells, prolonged exposure to homocysteine has been shown to induce unfolded protein response, resulting in cataract formation.[7,8] Homocystinuria (homocysteine > 100 μmol/L) is associated with congenital cataract[9,10] and lens dislocation/luxation,[9,10] but the association between higher serum homocysteine levels with age-related cataract is poorly understood.[6]

Vitamin B12 and folate are involved in homocysteine metabolism[11] and can lower homocysteine levels. Thus, in this study, we aimed to assess the cross-sectional association between posterior subcapsular cataract (PSC) and serum levels of homocysteine, vitamin B12, and folate in the second cross-sectional survey of the Blue Mountains Eye Study (BMES), and to validate any cross-sectional associations found longitudinally in the BMES cohort followed 5 years later.

Methods

The BMES is a population-based study of vision and other common eye diseases in an Australian population aged 49+ years, living in the Blue Mountains region, west of Sydney, Australia. The baseline examinations (BMES I) were conducted during 1992 to 1994 and recruited 3654 persons (82.4% of those eligible). Five years later, 2334 participants (75.1% of survivors) were re-examined in the second survey (1997–1999, BMES II). In addition, during 1999, newly eligible residents (who moved into the area or the age group) were recruited, and 1174 (85.2% of 1378 eligible) participated. Thus, the BMES

Table 1. Blue Mountains Eye Study Second Survey Population Characteristics by the Presence or Absence of Posterior Subcapsular Cataract at the Cross-Section II Survey (1997–2000)

Characteristics	No PSC Cataract, $n = 2494$	PSC Cataract, $n = 150$	P
Age, mean y (SD)	65.7 (9.0)	70.2 (8.1)	<0.0001
Female, %	57.1	54.0	0.4569
Current smokers, %	9.7	6.7	0.2252
Hypertension, %	75.6	82.6	0.0525
Diabetes, %	10.1	16.0	0.0212
Education, %*	65.3	54.0	0.0068
Myopia, %	15.3	34.7	<0.0001
Homocysteine, mean μmol/L (SD)	11.8 (4.2)	13.7 (7.9)	0.0039
Vitamin B12, mean pmol/L (SD)	286.7 (154.5)	280.8 (154.1)	0.6494
Folate, mean nmol/L (SD)	18.5 (9.0)	17.3 (8.2)	0.1150

* Education defined as trade certificate or higher qualification.

II cross-sectional survey population comprised of 3508 participants, 2334 original and 1174 newly recruited participants. Ten years after the BMES baseline, 1952 original cohort participants (76.6% of survivors) were re-examined for the third survey (2002–04, BMES III). For the purposes of this report, we used BMES II cross-sectional survey data to assess cross-sectional associations. For longitudinal associations, we used the BMES II cross-sectional survey as the baseline, when serum homocysteine, vitamin B12, and folate were measured. Incident PSC was detected at the BMES III survey to assess longitudinal associations.

There were 2644 participants of the BMES II cross-sectional survey with data available for the cross-sectional association analyses. We estimated our cross-sectional study sample would be able to detect a smallest odds ratio (OR) of 1.75 (with 80% power and α of 0.05) comparing the highest versus the lowest quintiles of homocysteine level. There were 1030 original cohort participants with follow-up information available for the longitudinal association analyses. We estimated that our longitudinal study sample would be able to detect a smallest OR of 3.7 comparing the highest versus the lowest quintiles of homocysteine level.

Detailed examination procedures have been described previously[12] and the same procedures were used for all examinations. Briefly, participants underwent a detailed eye examination, including lens photography, after pupil dilatation. Interviewer-administered questionnaires were used to collect medical and demographic information. The study adhered to the tenets of the Declaration of Helsinki, and was approved by the Human Ethics Committees of the University of Sydney and the Western Sydney Area Health Service. Written, informed consent was obtained from each participant.

PSC Grading

Detailed photographic and grading procedures have been described previously.[12] Briefly, lens photographs taken during the examinations were assessed for cataract in a masked manner by trained graders using the Wisconsin Cataract Grading System.[13] Retroillumination (Neitz CT-R; Neitz Instruments, Tokyo, Japan) photographs were used to determine PSC. Total area of involvement for PSC was estimated using a grid overlay. We defined PSC if any such opacity was present. Inter- and intragrader reliabilities for cataract grading were high and have been reported previously.[14]

Serum Assays

Detailed procedures on fasting blood have been described previously.[15] Briefly, fasting blood was collected at the study site, and tested at Westmead Hospital's Institute of Clinical Pathology and Medical Research the same day. Serum homocysteine assays were conducted on an IMx Analyzer (Abbott Laboratories, Abbott Park, IL, USA) using a fluorescence polarization immunoassay method. Serum vitamin B12 and folate assays were conducted on a Beckman-Access analyzer (Beckman Coulter, Inc., Fullerton, CA, USA) using a competitive-binding assay method.

Statistical Analysis

Analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA). We examined the association between serum levels of homocysteine, vitamin B12, and folate (study factors), and PSC prevalence and incidence (dichotomous outcomes). Multivariable-adjusted logistic regression models were used to estimate the associations, expressed as ORs and 95% confidence intervals (CI). Homocysteine was assessed continuously using the unit of each SD increase in serum levels, while vitamin B12 and folate were assessed continuously using the unit of each SD decrease in serum levels. The following potential confounders were included in the logistic regression model: age, sex, smoking status, hypertension, diabetes, education, myopia, and ever use of inhaled or oral steroids. We tested for interaction between homocysteine and vitamin B12 for PSC. When a significant interaction (effect modification, $P < 0.05$) was detected, we performed subgroup analyses stratified by the particular effect-modifying factor.

Results

PSC Prevalence

Of the 3508 participants, 493 did not have fasting blood samples collected. Of the remaining 3015 participants, 371 were excluded because of missing or ungradable photographs, or because cataract surgery had been performed previously. Thus, 2644 (75.4%) participants had data available for the cross-sectional association analysis.

Table 1 shows the BMES II study sample characteristics by cataract status. Participants with PSC tended to be older and were more likely to have a history of diabetes, myopia, and higher mean serum homocysteine levels than those without PSC; they also were less likely to have had higher education than those without PSC (Table 1).

The mean serum homocysteine level of this population was 12.2 μmol/L (SD, 4.8 μmol/L), mean serum vitamin B12 level was 285.4 pmol/L (SD, 154.6 pmol/L), and mean serum folate level was 18.3 nmol/L (SD, 9.0 nmol/L). In this population, PSC prevalence was 5.7% (150/2644) and there was a significantly higher prevalence of PSC among persons with elevated homocysteine (≥15 μmol/L) than those with normal homocysteine levels (8.4% vs. 5.3%, respectively, $P < 0.05$).

Table 2 shows the associations between homocysteine, vitamin B12, folate, and PSC prevalence. After multivariable adjustment, there was a 17% higher prevalence of PSC associated with each SD increase in serum homocysteine level (OR, 1.17; 95% CI, 1.00–1.37). There was a 24% higher prevalence of PSC associated with each SD decrease in serum folate (OR, 1.24; 95% CI, 0.99–1.56).

We found a significant interaction between homocysteine and vitamin B12 for PSC prevalence (interaction P value = 0.015). Table 3 presents the associations between homocysteine and PSC prevalence stratified by vitamin B12 level. When

Table 2. Associations Between Posterior Subcapsular Cataract and Serum Levels of Homocysteine, Vitamin B12, and Folate

	OR (95% CI) per SD Increase of Homocysteine	OR (95% CI) per SD Decrease of Vitamin B12	OR (95% CI) per SD Decrease of Folate
Prevalent PSC, $n = 150$			
SD	4.8 μmol/L	154.6 pmol/L	8.9 nmol/L
Age–sex adjusted	1.22 (1.06–1.40)	1.03 (0.88–1.22)	1.19 (1.00–1.43)
Multivariable adjusted*	1.17 (1.00–1.37)	1.06 (0.86–1.32)	1.24 (0.99–1.56)
Incident PSC, $n = 59$			
SD	4.1 μmol/L	144.0 pmol/L	8.6 nmol/L
Age–sex adjusted	0.94 (0.68–1.30)	1.24 (0.90–1.71)	0.88 (0.69–1.13)
Multivariable adjusted*	0.95 (0.69–1.32)	1.26 (0.90–1.77)	0.94 (0.72–1.23)

* Adjusted for age, sex, smoking status, hypertension, diabetes, education, myopia, and use of inhaled or oral steroids.

vitamin B12 was normal (≥125 pmol/L), there was a 28% increased odds of prevalent PSC associated with each SD increase in homocysteine (OR, 1.28; 95% CI, 1.09–1.52); in contrast, when vitamin B12 was low (<125 pmol/L), homocysteine seemed to be associated with reduced odds of prevalent PSC (OR, 0.16; 95% CI, 0.02–1.57, per SD increase in homocysteine), although this association was not statistically significant (Table 3).

PSC Incidence

The mean serum homocysteine level among BMES III participants with follow-up information ($n = 1030$) was 12.1 μmol/L (SD, 4.1 μmol/L), mean serum vitamin B12 level was 285.3 pmol/L (SD, 144.0 pmol/L), and mean serum folate level was 17.8 nmol/L (SD, 8.6 nmol/L). A total of 59 participants (5.7% of 1030) developed incident PSC. However, we found no longitudinal associations between baseline homocysteine, vitamin B12, folate, and incident PSC (Table 2).

Discussion

In this older Australian population, we observed a positive cross-sectional association between homocysteine and PSC prevalence, and an inverse cross-sectional association between folate and PSC prevalence. However, we could not confirm these associations using our longitudinal data from the same cohort, though the possibility of insufficient study power to detect longitudinal associations cannot be ruled out. Interestingly, we observed that vitamin B12 levels appeared to modify the cross-sectional association between homocysteine and PSC.

Table 3. Association Between Serum Homocysteine and Posterior Subcapsular Cataract Prevalence; Stratified by Vitamin B12 Levels

	PSC	
	n/N	OR (95% CI) per SD Increase of Homocysteine
Vitamin B12 ≥125 pmol/L	135/2395	
Age–sex adjusted		1.31 (1.13–1.52)
Multivariate adjusted*		1.28 (1.09–1.52)
Vitamin B12 <125 pmol/L	12/158	
Age–sex adjusted		0.58 (0.28–1.20)
Multivariate adjusted*		0.16 (0.02–1.57)

n/N, number of cases/number in group.
* Adjusted for age, sex, smoking status, hypertension, diabetes, education, myopia, and use of inhaled or oral steroids.

To our knowledge, only one other study has investigated the association between homocysteine and age-related cataract. Sen et al.[6] conducted a case-control study and found that there were significantly higher serum levels of homocysteine in cataract cases than controls, although the cataract type was not specified in the report, nor the methods used to ascertain the presence or absence of cataract. Cases and controls in the study by Sen et al.[6] were in a similar age group to our study sample (50+ years of age) and their study finding is consistent with ours.

Experimental studies have investigated the effect of homocysteine on human and animal lens epithelial cells,[8,16] specifically, the role of homocysteine in the unfolded protein response.[7] It has been suggested that homocysteine is a cataractogenic stressor that induces endoplasmic reticulum stress, triggering the unfolded protein response, reactive oxygen species generation, and proteolysis of lenticular proteins.[7,8,16] Human and rat lens epithelial cells exposed to high concentrations of homocysteine in vitro for a long period of time led to high percentage of lens epithelial cell death.[16] It was hypothesized that a strong unfolded protein response (for example, due to elevated serum homocysteine), which could generate large amounts of reactive oxygen species and could induce cell death and apoptosis, may result in the formation of cortical cataract.[16] The PSC formation from epithelial cells of the posterior lens capsule could occur in a similar way as cortical cataract when homocysteine concentration was high in the vitreous.

Congenital cataract has long been associated with homocystinuria.[9,17] It has been suggested that congenital cataract formation may be due to decreased bioavailablity of amino acids for lens formation or deficiency of the antioxidant glutathione.[17] Both causes are ultimately due to nutritional deficiencies in the fetus leading to high homocysteine levels. In age-related cataract cases, there could be a prolonged exposure to modestly elevated serum homocysteine, which could lead to a deficient antioxidant mechanism in the lens. Elanchezhian et al.[8] reported that incubation of human lens epithelial cells in lower concentrations of homocysteine for a prolonged period of time resulted in decreased antioxidant activity.

The increased risk for PSC with decreasing levels of folate could be related to the association between homocysteine and PSC, with folate being involved in the metabolism of homocysteine.[11] In the Framingham study, it was shown that participants with low levels of serum folate had correspondingly high levels of serum homocysteine.[18] Rat experiments showed that folate deficiency can increase homocysteine levels by 8- to 10-fold.[11] Conversely, many studies have shown that folate, either through a folate-rich diet or supplementation (with folic acid), can lower homocysteine levels effective-

ly.[19–23] Potentially, it may be possible to minimize risk of PSC by increasing dietary folate levels.

We could not confirm the cross-sectional association found between serum homocysteine and PSC in our longitudinal data. Approximately half of the original BMES cohort had follow-up data available ($n = 1030/2334$). Loss to follow-up due to mortality may explain the lack of association, as homocysteine is an independent predictor of all-cause mortality in this population.[24] Also, a previous comparison of participants and nonparticipants in BMES showed that nonparticipants were more likely to be smokers and to have diabetes,[25] both of which are important risk factors for PSC. Our cohort sample would only be able to detect a smallest OR of 3.7 when comparing the highest versus the lowest quintiles for longitudinal associations, which is double the smallest OR (1.8) that the cross-sectional analyses (150 prevalent PSC cases) can detect. For associations using per SD change in serum homocysteine level, the smallest OR that can be detected with only 59 incident cases also is likely to be double the OR of 1.2 detected in cross-sectional analyses.

The effect modification of vitamin B12 on the homocysteine-PSC association was unexpected. We cannot offer any biological explanations for the reduced prevalence in PSC for each SD increase in homocysteine in those with vitamin B12 levels <125 pmol/L. Caution is needed in interpretation, as the possibility of a chance finding cannot be ruled out.

The data for the subgroup with vitamin B12 ≥125 pmol/L is of concern as it suggests other causes of elevated homocysteine may be involved. Other causes of elevated homocysteine include kidney disease and hypothyroidism. In this population, kidney disease was common (20%)[26] and we have reported previously that raised plasma creatinine (an indicator of impaired kidney function) was associated with prevalence of PSC.[27] Prevalence of hypothyroidism was 4.1% in this population.[28] When excluding participants with raised creatinine and those with hypothyroidism from the analysis, the associations remained. Low serum folate level was associated with elevated homocysteine,[15] and inclusion of folate in the logistic regression models did not affect the association either (data not shown).

Nutrients known to be associated with elevated homocysteine include folate and vitamin B6.[18,29] It was suggested that adequate levels of three vitamins essential to homocysteine metabolism (i.e., folate and vitamins B6 and B12) may be needed for optimal levels of homocysteine[18,30] and that low levels of one or more of these vitamins could result in elevated levels of homocysteine.[18] We do not have serum measures nor dietary assessment of vitamin B6, and are unable to investigate this adequately.

Strengths of our study included its population-based sample, high baseline participation rate (78.2% of eligible), and the standardized methods used to examine participants and to assess PSC. However, there are some limitations. Although we adjusted for a number of known factors associated with cataract, other unidentified confounding factors may not have been accounted for in the models. We cannot exclude the possibility of chance findings, especially in the stratified subgroup analyses where the numbers in the subgroups were small. The inclusion of participants who had cataract surgery will provide more cases of PSC in analysis; however, we would highly likely introduce misclassification in the study outcome as we do not have information on cataract type before the surgery these participants had had. Loss to follow-up may have affected our ability to detect longitudinal associations. Finally, as our population was predominately Caucasian, these findings may not be generalizable to other racial groups.

In summary, in an older Australian population-based sample, we found that higher serum homocysteine was associated with a higher PSC prevalence, with vitamin B12 status possibly modifying this association. Lower serum folate was inversely associated with PSC prevalence. Future studies are needed to validate these observations.

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