Research Article

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Correlation between serum homocysteine, folate, vitamin B6 and age-related cataract

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Abstract: Age-related cataracts (ARC) are the leading cause of visual impairment and blindness, affecting 16 million subjects globally. This work aimed to investigate the correlation of serum homocysteine (Hcy), folate, vitamin B6 (VitB6) and ARC. We prospectively enrolled 60 ARC, and 58 age-matched healthy controls in this study. The serum concentrations of Hcy were determined using a fully automatic biochemical analyzer and folate/VitB6 by enzyme-linked immunosorbent assay (ELISA). The diagnostic performance of serum Hcy, folate and VitB6 for ARC were evaluated by receiver operating characteristics (ROC). The mean serum levels of Hcy, folate and VitB6 from the control group were $9.8 \pm 2.1 \mu\text{mol/L}$, $17.4 \pm 2.3 \text{ nmol/L}$, $42.3 \pm 5.7 \text{ pmol/L}$, respectively. In comparison, the mean serum levels of Hcy, folate and VitB6 from the ARC group were $12.2 \pm 2.5 \mu\text{mol/L}$, $15.3 \pm 2.6 \text{ nmol/L}$, $40.3 \pm 5.1 \text{ pmol/L}$, respectively. Significant statistical difference ($p < 0.05$) were found between the control and ARC groups. The diagnostic sensitivity, specificity and AUC of serum Hcy as a biomarker for ARC were 53.1%, 76.3% and 0.66 (95% CI: 0.61–0.76), respectively, which were superior to that of serum folate and VitB6. Serum Hcy was significantly elevated in ARC patients and correlated with ARC development, thus may be used as a serological marker for ARC diagnosis.

Keywords: Age-related cataract; homocysteine; folate; vitamin B6.

Introduction

Age-related cataract (ARC) refers to the opacity of the lens in middle-aged and elderly subjects [1]. With the increase of age, the incidence of cataract increases significantly [2]. The occurrence of this disease is related to the environment, nutrition, metabolism and heredity [3–5]. Oxidative stress induced by excessive reactive oxygen species (ROS) plays an important role in the development of ARC, and total homocysteine (Hcy) is considered to be one of the main inducers of excessive ROS formation. It has been reported that high plasma Hcy was an independent risk factor of posterior subcapsular ARC [6]. Folate, vitamin B6 (VitB6) and vitamin B12 (VitB12) are key enzymes, substrates and cofactors, which play a key role in the metabolism of Hcy [7]. In our present work, we investigate the correlation of serum Hcy, folate and VitB6 activity in ARC.

Materials and methods

Patient enrollment

We prospectively enrolled 60 ARC and 58 age-matched healthy controls in this study. All subjects underwent comprehensive ophthalmological examinations, including visual acuity, slit-lamp examination and fundoscopy. The ARC group consisted of those over 50 years of age with lens degeneration and opacity, and with the best corrected visual acuity being below 20/40, **Figure 1**. Patients were excluded using the following criteria: secondary cataract caused by trauma, glaucoma, ophthalmic pigmentitis and other causes, and systemic diseases such as diabetes, coronary heart disease, kidney disease and cancer. No cataract and ocular and systemic diseases were found in the healthy control group. The study was approved by the Tianjin First Central Hospital. The demographics of the included ARC patients and healthy controls were depicted in **Table 1**.

Informed consent: Informed consent has been obtained from all individuals included in this study

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Figure 1: Age-related cataracts. Immature cataracts (A, B, C) and mature cataract (D).

Table 1: Patient demographics for each group. Standard deviation (SD).

Group	Gender		Age (years)	
	Male	Female	Mean $\pm$ SD	Range
Control (n=58)	25	33	66.8 $\pm$ 4.8	55-85
ARC (n=60)	24	36	68.2 $\pm$ 7.6	51-86
Cortical (n=20)	7	13	69.3 $\pm$ 8.9	51-85
Nuclear (n=17)	10	7	67.2 $\pm$ 7.8	50-83
Posterior subcapsula (n=12)	5	7	68.6 $\pm$ 6.8	53-86
Mixed (n=11)	5	6	68.0 $\pm$ 7.1	52-85

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by Tianjin First Central Hospital's review board.

ARC and sub-types

According to the classification system of lens turbidity (Locs III), ARC was classified as nuclear (Locs III score > 4), cortical (Locs III score 2 - 4) or posterior subcapsular

(Locs III score < 2). There were two or more types of ARC defined as mixed ARC.

Serum Hcy, folate and VitB6 analysis

The serum concentrations of Hcy, folate and VitB6 were determined by a fully automatic biochemical analyzer or enzyme-linked immunosorbent assay (ELISA). Blood samples collected in EDTA anticoagulant were taken and centrifuged for 5 minutes at 2000r/min. The upper plasma was collected and stored in refrigerator at -80°C . Hcy concentrations were measured using a Hitachi 7020 automatic analyzer (Tokyo Hitachi Medical Group). The serum folate and VitB6 concentrations were determined using an ELISA kit in strict accordance with manufactures instructions.

Statistical analysis

STATA11.0 statistical software (<http://www.stata.com>) was used for data analysis. The measurement data were expressed as $\bar{x} \pm s$, and the comparisons between groups were made using the sample mean and t-test. The enumeration data were expressed using rate and the comparisons between groups were made using the chi-

Table 2: Mean serum Hcy, folate and vitamin B6 concentrations in control and ARC patients.

Group	Hcy ($\mu\text{mol/L}$)	Folate (nmol/L)	VitB6 (pmol/L)
Control (n=58)	9.8 $\pm$ 2.1	17.4 $\pm$ 2.3	42.3 $\pm$ 5.7
ARC (n=60)	12.2 $\pm$ 2.5	15.3 $\pm$ 2.6	40.3 $\pm$ 5.4
Cortical (n=20)	12.9 $\pm$ 2.8	15.8 $\pm$ 2.9	41.8 $\pm$ 5.6
Nuclear (n=17)	11.6 $\pm$ 2.1	14.2 $\pm$ 2.3	38.6 $\pm$ 4.9
Posterior subcapsula (n=12)	12.6 $\pm$ 1.9	14.8 $\pm$ 2.4	39.3 $\pm$ 5.4
Mixed (n=11)	11.8 $\pm$ 2.5	15.1 $\pm$ 3.1	42.4 $\pm$ 5.3

Table 3: Diagnostic performance of serum Hcy, folate and vitamin B6.

Diagnosis	Hcy	Folate	Vitamin B6
Sensitivity	53.1%	48.6%	50.3%
Specificity	76.3%	70.3%	42.1%
AUC(95%CI)	0.66(0.61-0.76)	0.55(0.46-0.60)	0.51(0.41-0.58)
p-value	<0.05	<0.05	<0.05

square test. The diagnostic sensitivity, specificity, and the area under the ROC curve were calculated according to the Bayes' theorem. A P-value of less than 0.05 equated to statistical difference.

Results

Serum Hcy, folate and VitB6 concentrations

The serum Hcy, folate and VitB6 concentrations from the control, ARC and sub-type groups were displayed in **Table 1**. There were significant statistical differences between serum Hcy, folate and VitB6 between control and ARC groups ($p < 0.05$), **Table 2**.

Diagnostic performance of serum Hcy, folate and VitB6 for ARC

The diagnostic sensitivity, specificity and AUC of serum Hcy, folate and VitB6 as serological markers for ARC were shown in **Table 3**. The diagnostic performance of Hcy was superior to folate and VitB6.

Discussion

Cataracts are the leading cause of blindness worldwide [8]. In the US, age-related lens changes have been reported

in 42% of those between the ages of 52 and 64 [9], 60% between the ages 65 and 74 [10], and 91% between the ages of 75 and 85 [9]. The development of cataracts are the result of a combination of factors, such as radiation and free radical damage; lack of nutrients; chemicals; antibiotics; metabolic disorders such as glucose, galactose, lipid peroxidation product damage [11-14].

It has been reported previously that high Hcy initiated unfolded protein response (UPR) and weakened the intraocular oxidative defence system [15]. High levels Hcy were closely related to juvenile cataracts and age-related cataracts. Sen et al. [16], found that serum levels of Hcy in cataract patients were significantly higher than those in the age-matched control group, and the increased levels were related to a reduction in folic acid and VitB12 levels. Tan et al. [6], investigated the serum levels of Hcy in 3508 subjects with an average age of 65 and found that the serum levels of subcapsular cataract patients were significantly higher than those in the control group. High concentrations of Hcy can induce severe oxidative stress in lens cells and induce the occurrence and development of cataracts. The increase of Hcy leads to a large number of ROS, cell death and apoptosis, which may lead to the formation of cortical cataracts [17]. The development of posterior subcapsular cataract may be related to the increase of Hcy in the vitreous [18].

Folate, VitB6 and VitB12 are substrates and cofactors of Hcy metabolism and correlate to Hcy levels [19]. Previous studies have found that serum folate levels decreased by one standard deviation and the risk of posterior subcapsular ARC increases by 24% [6]. According to previous publications, the correlation between high Hcy levels and ARC have been shown [6, 16, 20]. However, the diagnostic performance of serum Hcy as a biomarker in the prediction of ARC was not widely discussed. In our study, we investigated the diagnostic performance of serum Hcy, folate and VitB6 as serological markers for the prediction of ARC. We found that serum Hcy was significantly elevated in ARC patients and correlated with

ARC development and thus can be used as a serological marker for ARC diagnosis.

However, there were several limitations of this study, such as small sample size and single centre patient recruitment, which may have caused limited statistical power and subject inclusion bias. In addition, this study did not consider the effect of dietary vitamins and folic acid on the results. Therefore, a larger sample size with more comprehensive experimental design studies is necessary to further verify the predictive value of these plasma indicators for ARC.

Conflict of interest: Authors state no conflict of interest

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