·Clinical Research·

Matrix γ-carboxyglutamate protein and Fetuin-A, in wet type age-related macular degeneration

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Abstract

• AIM: To evaluate the high sensitivity C-reactive protein (hsCRP), Fetuin -A and matrix γ -carboxyglutamate protein (MGP) as the main factors for vascular calcification and inflammation in serum of patients with advanced age-related macular degeneration (ARMD) in comparison to healthy controls.

• METHODS: The subjects were 40 patients with choroidal neovascularization (CNV) having a mean age of 70.9±9.1y and a matched group of 49 apparently healthy control subjects. The ARMD was diagnosed using a slit-lamp with superfield lens, fundus photography and fluorescein angiography. Measurement of hsCRP was done by nephelometry method. Levels of Fetuin-A and MGP were measured by enzyme-linked immunosorbent assay (ELISA) technique.

• RESULTS: hsCRP [0.45(0.07-2.63) mg/L *vs* 0.25(0.03-1.2) mg/L, *P*=0.02)] and Fetuin-A levels (50.27±5.04 *vs* 44.99±10.28 ng/mL, *P*=0.009) were higher in the patients than in the control groups. We could not find significant difference in MGP level between two groups (*P*=0.08). There was not a significant correlation between MGP with Fetuin-A and hsCRP among the patients (*P*=0.7, *P*=0.9 respectively). A significant negative correlation of hsCRP with Fetuin-A was observed in both case and control groups (*P*=0.004, *r*=-0.33 and *P*=0.001, *r*=-0.54, respectively).

• CONCLUSION: Although our study shows that serum hsCRP and Fetuin-A is increased in CNV patients as well as negatively correlated with both study groups, their direct role on pathogenesis of ARMD required future studies.

• **KEYWORDS:** high sensitivity C-reactive protein; Fetuin-A; matrix γ-carboxyglutamate protein; age-related macular degeneration; choroidal neovascularization
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INTRODUCTION

Age-related macular degeneration (ARMD) is a common cause of severe visual loss in elder people. There are two forms of ARMD, non-exudative and exudative. To date no single etiology has been identified as causative of exudative ARMD. Multiple factors have been proposed as promoters of new blood vessels formation in wet ARMD[1]. However, it is thought that inflammatory processes contribute to both drusen formation and choroidal neovascularization (CNV) in ARMD[2]. Proposed theories to explain the stimulus for such inflammatory cascades include excessive oxidative stress, dietary deficiency, light exposure, and autoimmune mechanisms[3-5].

Mitta *et al*[6] found that increased high sensitivity C-reactive protein (hsCRP) level is an independent risk factor for ARMD.

The glycoprotein Fetuin-A, also referred to as α_2-Heremans-Schmid glycoprotein, is almost exclusively expressed and secreted by the liver, particularly under hepatic steatosis [7]. Fetuin-A is a natural inhibitor of the insulin-stimulated insulin receptor tyrosine kinase and was shown to induce insulin resistance in rodents [8-11]. Studies in humans have demonstrated that circulating Fetuin-A levels are positively associated with fat accumulation in the liver, insulin resistance, and the metabolic syndrome [7,12,13]. Recently, two independent prospective cohort studies have demonstrated that Fetuin-A is positively associated with risk of type II diabetes mellitus[14,15]. In addition to the induction of insulin resistance, recent data suggest that Fetuin-A is involved in subclinical inflammation. Circulating Fetuin-A correlates positively with hsCRP level in humans [13,15]. Furthermore, Fetuin-A was recently found to induce cytokine expression in human monocytes[16].

However, it is unclear whether Fetuin-A is associated with CNV in humans. Therefore, we investigated the relationship between plasma levels of Fetuin-A and exudative ARMD or CNV.

Matrix γ-carboxyglutamate protein (MGP) is expressed in the media of arteries where it acts as a local inhibitor of Ca-PO_4 precipitation[17]. Circulating Fetuin-A and MGP levels have been linked with cardiovascular mortality in adults with

chronic kidney disease (CKD), but there is a complex and poorly understood relationship between these physiological calcification inhibitors at different stages of CKD and also contradictory data on their impact on vascular events[18-22].

The compositional similarity between drusen and other disease deposits may be significant in view of the recently established correlation between ARMD and atherosclerosis. The research study suggests that similar pathways may be involved in the etiologies of ARMD and other age-related diseases [23]. Drusen associated with aging and ARMD contains proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease[24].

Based on the literature, the aim of the present study was to evaluate hsCRP, Fetuin-A a biomarker correlated with atherosclerosis and cardiovascular diseases and MGP as the main factors for vascular calcification in the sera of the patients with exudative ARMD and to compare them with healthy controls.

SUBJECTS AND METHODS

Subjects This study was designed as a prospective case-control study and was performed from December 2010 to January 2012 in the Nikokari Eye Hospital in Tabriz, Iran. Informed consent form was filled by all of the patients, and the study was approved by the ethical committee of Tabriz University of Medical Science and was conducted in accordance with the ethical principle outlined in the Declaration of Helsinki.

We selected 40 CNV cases and 49 healthy controls. All the patients underwent a detailed ophthalmic examination, including dilated fundus examination and fundus photography. The fluorescein angiography was performed in ARMD patients for diagnosis of CNV.

Height and weight were measured at baseline to calculate body mass index (BMI). Participants were excluded from the study for a history of heavy smoking, major systemic disease (diabetes mellitus, dyslipidemia, renal or hepatic disease, hematologic and autoimmune disorders and arteriosclerotic disease) and any history of special drug usage (*e.g.* Coumadin and chemotherapeutic agent) that could influence the MGP, hsCRP and Fetuin-A. In addition participants with any evidence of macular disease other than ARMD, secondary CNV diseases such as ocular trauma, presumed ocular histoplasmosis, degenerative high myopia, retinal detachment, chorioretinal infective or inflammatory processes, angioid streaks, and cases of large cicatricial lesions, and any retinopathy associated with ischemia and intraoclar neovascularization were excluded from the study. Patients were included in the study with subfoveal CNV due to ARMD and age range of 40-85y.

Methods

Sample collection and analysis All the participants underwent blood sampling after 8h fasting. All samplings were performed in a period of 4wk. The separated plasma and serums were collected and stored in -70℃ until laboratory tests were done.

Laboratory methods Serum MGP concentration was measured by using a novel commercially available enzyme-linked immunosorbent assay (ELISA) kit (TSZ ELISA-Cat NO. HU8370, 185 Wilson Street, Framingham, MA 01702, USA) with a detection range of 15-500 ng/mL. The standard curve concentrations used for the ELISA's were 500, 250, 125, 62.5, 31.25 and 0 ng/mL. Serum total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic colorimetric method with an automated chemical analyzer (Abbott analyzer, Abbott laboratories, Abbott Park, North Chicago, IL, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated by using the Friedewald formula. Serum concentrations of Fetuin-A were measured using a human Fetuin-A ELISA kit in an ELISA plate reader (STATFAX2100, Multi-detection Multi Plate Reader, USA). Fetuin-A concentration was determined by interpolation with a standard curve. The analytical limit detection of the assay was 0.35 ng/mL, with inter-assay coefficient of variation (CV) of 6.5% and intra-assay CV of 5.1% (BioVendor Laboratory Medicine Inc., Brno, Czech Republic). hsCRP was measured by nephelometry method (Pars Azmoon Co.).

Statistical Analysis Statistical analysis was performed using SPSS version 13. Values were expressed as median (minimum-maximum values) for non-parametric and mean± standard deviation for parametric analysis. Differences among groups were assessed by Mann-Whitney *U* test for the non-parametric data or independent sample *t*-test for parametric data and also Spearman coefficient was calculated to determine the correlation between biochemical parameters. $P<0.05$ was considered statistically significant.

RESULTS

The mean age of CNV patients and controls was 70.93±9.12y and 69.57±7.39y, respectively. All of the patients completed the study and none were excluded. All measurements were performed successfully, without any failure in determining Fetuin-A, CRP and MGP owing to insufficient sampling or any other complication during the analysis. Demographic and baseline characteristics of the patients are shown in Table 1.

Two groups were adjusted in terms of age, sex, and other variables including blood glucose and lipid profile. Although mean weight of both groups was not similar but there was not any significant difference in BMI.

Mean Fetuin-A was significantly higher among 40 CNV patients compared to 49 healthy controls. The CRP levels were significantly higher among participants with CNV (case patients) than those with no CNV (controls). Mean MGP levels were not significantly higher in controls than those in CNV patients (Table 2).

As far as hsCRP and Fetuin-A are concerned we found significant negative correlation between them in both cases and control groups as shown in Table 3.

Table 1 Demographic and biochemical characteristics of patients with exudative armdand controls

Variables	Patients *n*=40	Controls *n*=49	*P*
Age (a)	70.93±9.12	69.57±7.39	0.54
M/F	20/20	24/25	0.94
Height (cm)	163.35±9.44	165.35±7.47	0.17
Weight (kg)	69.15±13.03	72.43±7.80	0.003
FBS (mg/dL)	91.80±9.97	93.20±11.47	0.73
TC (mg/dL)	184.00±35.42	181.63±36.77	0.91
HDL-C (mg/dL)	44.51±2.72	44.16±0.53	0.37
LDL-C (mg/dL)	113.25±36.27	103.57±34.37	0.37
TG (mg/dL)	140±65	148±69	0.39
BMI (kg/m^2)	24.3±0.4	25.1±0.5	0.30

FBS: Fasting blood sugar; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglyceride; BMI: Body mass index.

Table 2 Fetuin-A, hsCRP and MGP in patients with exudative ARMD and controls

Variables	Patients	Controls	[1]*P*
Fetuin-A (ng/L)	50.27 (45.23-55.31)	44.96 (34.48-45.50)	0.009
MGP (Pg/mL)	32.99 (7.71-58.27)	40.5 (10.90-70.14)	0.075
hsCRP (mg/L)	0.45 (0.07-2.63)	0.25 (0.03-1.20)	0.020

hsCRP: High sensitivity C-reactive protein; MGP: Matrix γ-carboxyglutamate protein. [1]Performed by Mann-Whiney *U* test.

Table 3 Correlation between Fetuin-A, hsCRP, and MGP among study groups

Variables	*P*	*r*
Correlation[1] in CNV patients (*n*=40)		
Fetuin-A with hsCRP	0.004	[2]-0.330
Fetuin-A with MGP	0.70	-0.66
MGP with hsCRP	-0.90	-0.924
Correlation[1] in Controls (*n*=49)		
Fetuin-A with hsCRP	0.001	[2]-0.540
Fetuin-A with MGP	0.636	-0.69
MGP with hsCRP	0.14	0.212

hsCRP: High sensitivity C-reactive protein; MGP: Matrix γ-carboxyglutamate protein. [1]Performed by Spearman´s test; [2]Correlation is significant at the $P<0.05$.

DISCUSSION

To the best of the authors' knowledge, this is the first report to assess the MGP, Fetuin-A and hsCRP in CNV patients. In the present study, we found that plasma Fetuin-A levels are higher than normal in exudative ARMD. These data support the hypothesis that Fetuin-A may play a role in the pathophysiology of CNV, which may be independent of the classic risk factors. Previous studies revealed that Fetuin-A is elevated in the case of humans with fatty liver and the metabolic syndrome [7,12,13]. These conditions are strongly associated with insulin resistance [25]. Fetuin-A itself was found to inhibit the insulin receptor kinase, thus inducing insulin resistance in animals[9,11]. In humans, Fetuin-A plasma levels predicted insulin resistance, measured by the euglycemic hyperinsulinemic clamp [7,12] and were positively associated with risk of type II diabetes mellitus [14,15]. The studies suggested a possible positive association between diabetes and/or hyperglycemia with ARMD [26,27]. Thus, Fetuin-A may promote ARMD *via* induction of insulin resistance.

Fetuin-A may play also a role in the pathophysiology of subclinical inflammation, thereby affecting CNV risk. This hypothesis is supported by recent findings that Fetuin-A promotes cytokine expression in human monocytes[16].

Studies have suggested that inflammation plays a role in the pathogenesis of drusen and ARMD [28,29]. Drusens contain proteins that are associated with chronic and acute inflammatory responses [30] and other age-related diseases, including amyloid P component and complement proteins[31]. Inflammation is also associated with angiogenesis, and may play a role in the neovascularization seen in the advanced form of ARMD.

Our study demonstrated that serum levels of the systemic inflammatory markers and hsCRP are significantly elevated in individuals with advanced ARMD. In stratified analyses, the highest levels of hsCRP were associated with a twofold increased risk of ARMD among both smokers and nonsmokers [28]. These elevated levels suggest that reducing inflammation may slow the progression of ARMD.

In addition, we found a significant inverse association between Fetuin-A and hsCRP in CNV patients. Further studies are needed to investigate the role of Fetuin-A in the CNV patients.

Although MGP level were lower in CNV patients than that of the controls, we could not find any correlation between MGP and CNV.

Among the strengths of our study is its prospective design. Nevertheless, some limitations of our study should be noted. Relative mall sample size is one of them. Our results are based on Fetuin-A measurements from single blood samples, which might have introduced random measurement errors in determining biochemical variables. However, if anything, such random error would bias the results towards the null. The potential of residual confounding applies to our study as it does to observational studies in general. Although we adjusted for a large variety of known risk factors and biochemical variables, we cannot rule out that unmeasured factors explain our observation. At last, this novel finding might have prognostic or diagnostic values however the observed association needs to be supported by a prospective study. Prospective investigations in the future would be helpful to fully define this finding's application.

In conclusion, the present pivot and preliminary study suggests high levels of Fetuin-A, a serum biomarker, in exudative ARMD patients. Additional prospective studies are needed to further elucidate the exact roles and applications.

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