ORIGINAL ARTICLE

Serum APOE, leptin, CFH and HTRA1 levels in Pakistani age related macular degeneration patients

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Abstract

Objective: To determine the association between serum levels of apolipoprotein E, leptin, complimentary factor H and high temperature requirement A-1 in patients with age-related macular degeneration.

Methods: This case-control study was conducted at the Quaid-i-Azam University, Islamabad, Pakistan, from May to October 2013, and comprised patients with age-related macular degeneration and matching controls. The confirmation of age-related macular degeneration was carried out through slit lamp examination, fundoscopy and ocular coherence tomography. The selected subjects were not suffering with any other systemic or ophthalmic complication(s). Serum apolipoprotein E, leptin, complimentary factor H and high temperature requirement A-1 were estimated in serum samples of all subjects. SPSS 18 was used for data analysis.

Results: Of the 190 participants, 90(47.4%) were patients with age-related macular degeneration and 100(52.6%) were controls. Significantly elevated serum apolipoprotein E ($p<0.0024$) and high temperature requirement A-1 ($p<0.0001$) levels were observed in the patients, while serum leptin ($p<0.008$) and complimentary factor H ($p<0.0001$) levels were significantly reduced. Logistic regression showed that lower leptin ($p<0.026$) and elevated high temperature requirement A-1 ($p<0.0001$) were the relevant risk factors.

Conclusion: Serum apolipoprotein E, leptin, complimentary factor H and high temperature requirement A-1 levels were altered in age-related macular degeneration patients.

Keywords: AMD, APOE, Leptin, CFH, HTRA1. (JPMA 67: 852; 2017)

Introduction

One of the main causes of irreversible blindness in people aged 50 years or above is age-related macular degeneration (AMD). This increases the burden of aged blinds in the society. Although treatment options for AMD are becoming available, prevention of the disease through public health awareness is also important.[1] Over the past few years, extensive studies have been done on the aetiology of AMD, many of which focused on discovering the risk factors related to the disease. Cardiovascular diseases, high lipid content, obesity and cigarette smoking are well known risk factors for AMD.[2] Besides, the role of inflammation in the pathogenesis of AMD has also been indicated, but the role of immune system in this disease has yet to be fully understood. It has, however, been suggested that large and soft drusen, an important symptom of AMD, are good indicators of immune-mediated local inflammatory processes in the eye.[3] Recent data on AMD provides evidence that inflammatory processes and oxidative stress are also involved in the pathogenesis of AMD.[4]

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From the diagnostic point of view, choroidal neovascularisation (CNV) is an important symptom of AMD. It is suggested that neovascularisation and angiogenesis are associated with inflammation in ocular diseases like AMD as the inflammatory response acting through the macrophage component promotes anomalous angiogenesis in CNV.[5]

Apolipoprotein E (APOE) has been shown to modulate oxidation and inflammation. Its function is linked with the anti-inflammatory and pro-inflammatory cytokines. Correspondingly, the APOE production in various tissue types is upregulated or downregulated by inflammatory cytokines.[6] The APOE suppresses vascular endothelial growth factor (VEGF) and chemokine expression in retinal pigment epithelial (RPE) cells. However, this influence appears to be isoform dependent as APOE4 is more potent in its action than APOE3.[7] Leptin is another angiogenic factor that induces CNV. It is also involved in metabolism, neuroendocrine physiology, immune function and development.[8]

Complementary factor H (CFH) is a glycoprotein that plays critical role in the regulation of complex immune-mediated processing and apoptosis.[9] Single-nucleotide polymorphisms (SNPs) and mutations in the CFH gene appear to be causative factors behind AMD and many

other inflammatory diseases that include membranoproliferative glomerulonephritis and atypical haemolytic uremic syndrome.[10] The single-nucleotide polymorphism Y402H in the CFH gene has been studied to be associated with drusen and pigmentary changes in the eye indicating prior ocular inflammation. These variants show reduced binding to C-reactive protein (CRP) and heparin. CRP is one of the inflammatory markers that play a pivotal role in atherosclerosis and endothelial cell dysfunction.[11]

High temperature requirement A-1 (HTRA1), a member of HTRA family of chymotrypsin-like serine proteases, shows temperature-dependent proteolytic activities. It has a C-terminal HTRA domain and N-terminal secretory signal peptide which is a secreted enzyme that cleaves substrates like vitronectin, fibromodulin and clusterin.[12] HTRA1 is a secreted protein and is known to be upregulated in osteoarthritis and down regulated in transformed fibroblasts in ovarian cancer and melanoma.[13] HTRA1 has been suggested to be involved in the pathogenesis of AMD. HTRA1 allele rs10490924 T, present in the promoter region of gene, is associated with increased HTRA1 expression.[14] A link between HTRA1, complement regulation and amyloid deposition has also been suggested in AMD pathogenesis.[15]

AMD is usually considered as chronic, age-related inflammatory disease. However, no concrete data exists from the point of view of above factors in Pakistani population. The current study was planned to determine alterations and association among serum inflammatory and angiogenic factors, namely the APOE, leptin, CFH and HTRA1 in AMD patients.

Patients and Methods

This case-control study was conducted at the Quaid-i-Azam University (QAU), Islamabad, Pakistan, from May to October 2013, and comprised AMD patients and controls. All the procedures were followed in accordance with the latest version of Helsinki Declaration.[16] The study was formally approved by the institutional ethics committee. Written informed consent was obtained from all participants after narrating to each one of them the study procedures. History of the disease was recorded for each patient after the confirmation of AMD diagnosis through slit lamp examination (C-153 TOPCON, SL 3D, United States), ocular coherent tomography (OCT) (Zeiss, Germany) and fluorescent fundus angiography (FFA) (Topcon, TRC50EX, retinal camera, Image Mat, 2000, US). Control subjects also underwent detailed ocular and physical examination for the confirmation of healthy conditions. Blood glucose level and blood pressure were calculated and complete medical history was recorded in all the subjects. Vision of all the subjects was recorded through Snellen chart examination. Subjects of both genders aged $\geq$50 years, non-diabetic, non-hypertensive with no complications of the eye other than AMD were included. Control subjects did not show any signs and symptoms of AMD. Similar were the inclusion criteria for control subjects. Wet AMD was diagnosed in the patients having CNV with exudation while in dry type AMD no exudative CNV was observed while atrophic changes were present.

Cubital vein blood (3ml) was drawn to prepare serum. Serum samples were aliquoted and kept at -20^{o}C until analysed. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used for the estimation of different proteins. These included: APOE (Assaypro, US), Leptin (Assaypro, US), CFH (Cusabio, China) and HTRA1 (ElAab, China). Analyses were carried out on a fully automated microplate reader (KHB-ST 360, China) with automatic plate washer from KHB-ST-36W (China). The assays were run according to the manufacturer's instructions. All the assays were run in duplicate.

SPSS 18 and Graph Pad Prism version 5.00 for Windows (GraphPad Software, San Diego California, US) were used for data analysis. Student's t-test was applied to compare protein concentrations in the diseased and control groups.

Logistic analysis, a predictive analysis when the dependent variable is dichotomous, was used. It is used to explain the relationship between one dependent binary variable and one or more than one variables.[17] In the current study, logistic regression analyses predicted if changes in serum proteins were related to increased risk of disease. $P<0.05$ was considered significant. The data was transformed using the method described by Box and Cox[18] for reducing heterogeneity of error that permitted the assumption of equal variance to be met. Box and Cox transformation provided an algorithm through which the optimal value of the transformation parameter λ was selected by the method of maximum likelihood. Transformed data was subjected to multivariate analysis of variance (M-ANOVA) for statistical evaluation of the association of serum APOE, leptin, CFH and HTRA1 levels with AMD.

Results

Of the 3,911 patients screened for ophthalmic complications, 90(2.3%) were diagnosed with AMD. Besides, 100 age-matched control subjects were included. Control subjects included 45(45%) females and 55(55%) males, while among AMD patients, 59(65.6%) were males

Table: Logistic regression in Control and AMD patients.

Proteins	B	Wald	Sig.	Exp(B)
Comparison of controls to AMD patients				
Leptin	1.642	0.739	0.026*	5.164
HTRA1	0.001	0.000	0.0001***	1.001
Comparison of control males to AMD males				
HTRA1	0.001	6.359	0.012*	1.001
Comparison of control females to AMD females				
APOE	0.308	4.676	0.031*	1.36
HTRA1	0.001	4.882	0.027*	1.00
Comparison of Wet AMD with dry AMD				
Leptin	1.497	4.296	0.038*	4.47

*** p<0.0001; *p<0.05
AMD: Age-related macular degeneration
APOE: Apolipoprotein E.

and 31(34.4%) were females. Among AMD patients, 63(70%) had wet type and 27(30%) had dry type AMD. Small drusen were observed in all AMD patients whereas large drusen were observed in 80(88.9%), including 58(72.5%) wet and 22(27.5%) dry AMD patients. RPE changes were evident in 80(88.9%) (57(71.25%) wet and 23(28.75%) dry AMD) patients. CNV was present in 65(72.2%) AMD patients (54(83%) wet and 11(17%) dry). Geographic atrophy (GA) was observed in 33(36.7%) AMD patients (16(48.5%) wet and 17(51.5%) dry).

Serum APOE concentration was significantly elevated (p<0.0024) in AMD patients as compared to the control subjects. In male patients with AMD, APOE concentration was not different from the healthy males of control group (p<0.2507). In female AMD patients, APOE levels were significantly elevated (p<0.011) in cases as compared to controls. In patients with dry AMD, the APOE concentration was not (p<0.6475) different from the patients with wet AMD (Figure: A).

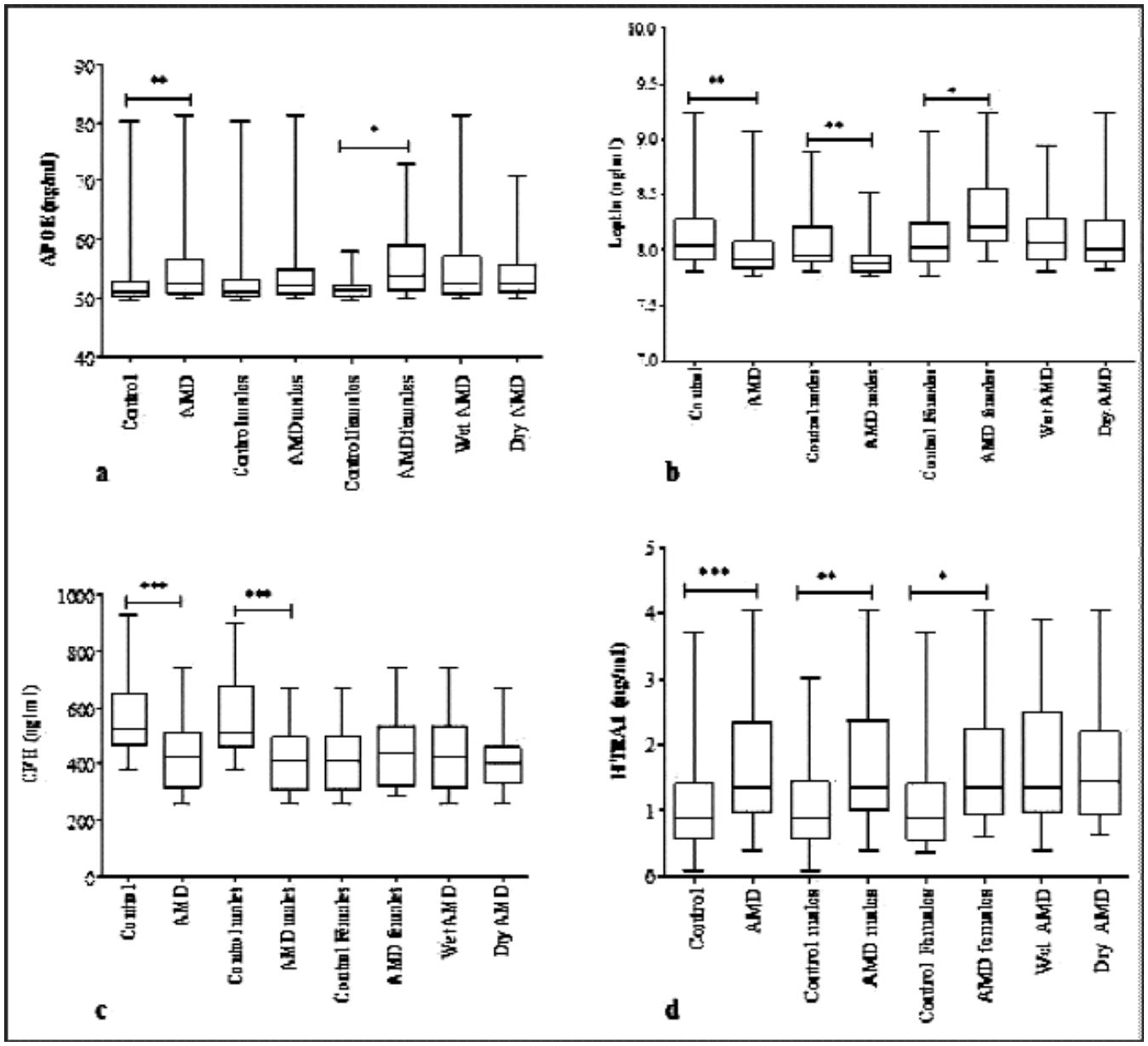
The concentration of serum leptin was significantly lowered (p<0.008) in patients with AMD as compared to the healthy control subjects. In males, leptin concentration was significantly (p<0.0009) reduced in cases as compared to controls group. In female AMD patients, the serum leptin levels were significantly reduced than control females (p<0.017). Leptin concentration did not differ significantly between wet and dry AMD (p<0.8735) (Figure: B).

The serum CFH levels in the patients with AMD were significantly reduced compared to control group (p<0.0001). In males this difference was significant (p<0.0001), but there was no significant difference in female cases and controls (p<0.3008). The serum CFH levels in patients with wet AMD were non-significantly (p<0.2757) higher compared to the patients with dry AMD (Figure: C).

In patients with AMD, HTRA1 serum levels were significantly elevated (p<0.0001) compared to control subjects. In male AMD patients, the HTRA1 concentration increased significantly (p<0.0005) compared to male controls. The same pattern was found with female cases and controls (p<0.0162). There was no difference in serum HTRA1 levels between patients with dry AMD and wet AMD (p<0.7475) (Figure: D).

Box and Cox transformation provided an algorithm through which the optimal value of the transformation parameter λ was selected by the method of maximum likelihood. The analysis of variance for λ= 0.175 that yield the lowest error sums of squares was then used for hypothesis testing. Transformed data showed that serum APOE concentration was significantly lower in AMD patients compared to that in control subjects (p<0.0032). These levels were not found to be associated with the gender (p<0.843; Wilk's λ= 0.999; F= 0.170). However, serum levels of leptin, CFH and HTRA1 were not significantly changed in AMD patients compared to control subjects as analysed after Box and Cox transformation.

The logistic regression analyses between control and AMD patients showed leptin (p<0.026) and HTRA1 (p<0.0001) to be the risk factors involved in AMD progression. Omnibus tests had chi-square value 27.979 (p<0.0001; Cox and Snell R-squared 0.192; Nagelkerke's R-squared 0.256). Logistic regression analyses of male AMD patients with male control subjects revealed that HTRA1 (p<0.012) to be the risk factors. Omnibus tests had chi-square value 12.667 (p<0.013; Cox and Snell R-squared 0.148; Nagelkerke's R-squared 0.198). In female patients



AMD: Age-related macular degeneration. APOE: Apolipoprotein E. CFH: Complementary factor H.

Figure (A-D): Box and Whisker plots showing concentrations of (a) APOE (b) leptin (c) CFH (d) and HTRA1 in AMD patients and controls, males and females and wet and dry AMD patient group. Bars represent median value and whiskers represent minimum and maximum values. p values are based on Student's t-test.*** p<0.0001; **p<0.01; *p<0.05.

with AMD, APOE (p<0.031), HTRA1 (p<0.027) were predicted to be the risk factors. Omnibus tests had chi-square value of 22.917 (p<0.0001; Cox and Snell R-squared 0.356; Nagelkerke's R-squared 0.475). In comparisons based on AMD type, reduced leptin level was the risk factor (p<0.031). Omnibus tests had chi-square value of 4.812 (p<0.0307; Cox and Snell R-squared 0.036; Nagelkerke's R-squared 0.055) (Table).

Discussion

The present study provides evidence that elevated serum APOE and HTRA1 levels and reduced leptin and CFH levels are related to AMD. The present study adds further to the pathology of AMD by showing the involvement of inflammatory processes. The current study provides first direct evidence of changes in serum levels of inflammatory markers like APOE, leptin, CFH and HTRA1

in AMD patients in comparison to age-related control subjects. The inflammatory proteins play contributory role in the progression of the disease that results into increased number of drusen, GA and CNV, all leading to complete loss of central vision.[19,20]

Presently, APOE levels were found elevated in AMD patients. Similar were the observations in a study on French AMD patients.[21] Lipoproteins that pass through and accumulate in RPE and Bruch's membrane provide substrate for initial changes of AMD-like drusen formation. These deposits also provide substrate for chronic inflammation. In addition, APOE is also a component of drusen.[22] The underlying mechanism of APOE might be the altered regulation of VEGF in RPE cells by APOE isoforms.[23]

In the current study, leptin levels were significantly reduced in AMD patients compared to normal controls. A recent study from Singapore has reported an inverse relationship of leptin levels to AMD ($p<0.005$).[24] Leptin has a direct metabolic effect on lipoprotein metabolism or lipase activity. In case of leptin deficiency, fatty acid synthesis would increase due to the inhibition of mitochondrial fatty acid uptake and oxidation resulting in elevated intracellular fatty acids and triglyceride levels. The intracellular lipid accumulation in RPE leads to AMD that appears in the form of Bruch's membrane deposits and drusen.[25]

In the present study, serum CFH levels were significantly reduced in AMD patient group. CFH is one of the regulators of inflammatory pathways; it prevents the formation of C3 convertase enzyme and enhances its dissociation. Reduced CFH concentration is likely associated with other risk factors for AMD such as hypertension and obesity.[26]

It has been suggested that insufficient CFH at retina or choroid may lead to uncontrolled complement activation associated with cell and tissue damage. On the contrary in another study, plasma CRP levels were not changed in the AMD patients compared to controls and were not influenced by the CFH SNP (Y402H) or the age. This SNP in the SCR7 domain of CFH causes reduced binding of CFH to CRP, which results to high levels of unbound CRP and leads to chronic inflammation.[27]

Conclusion

Changes in serum levels of APOE, leptin, CFH and HTRA1 were found related to AMD pathogenesis. The current study was the first report from Pakistan showing changes in serum levels of the inflammatory markers. It is suggested that AMD is in fact an inflammatory pathology that involves a number of processes leading to inflammation and angiogenesis. It is predicted that the therapeutic agents focusing specific complement-modulation agents and non-specific anti-inflammatory drugs might be effective to treat AMD.

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