

Atherosclerosis impacts the link between hepatocyte growth factor and cognition

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Background. There is a controversy about the association between Hepatocyte growth factor (HGF) and cognition. Increased serum level of HGF has been reported in patients with hypertension, peripheral arteriosclerosis and carotid atherosclerosis. Ankle-brachial index (ABI) is considered as a marker of atherosclerosis. we hypothesized that hypertension or atherosclerosis with hypertension could alter the relation between HGF serum level and cognitive function.

Aim. To study HGF and cognitive function in hypertensives with and without atherosclerosis versus healthy controls.

Methods. This case-control study included ninety elderly subjects attending outpatient primary care geriatric clinics. They were subdivided into 3 groups; Group A (30 normotensives with normal Ankle-brachial index (ABI) as controls, group B (30 hypertensives with normal ABI) and group C (30 hypertensives with abnormal ABI) as cases. Cognitive function was assessed by Rowland Universal Dementia Assessment Scale (RUDAS).

Results. Group C had worse score in RUDAS than controls ($P = 0.01$). HGF was negatively correlated with ABI in group C ($p = 0.007$). HGF was positively associated with RUDAS score, in group A ($p < 0.001$), in group B; after further adjustment for systolic blood pressure (SBP) ($p = 0.024$) and in group C; after adjustment for ABI ($p = 0.031$) or ABI and SBP ($p = 0.05$).

Conclusions. The potential beneficial link between HGF serum concentration and cognition was met in normotensive subjects with normal ABI. However, this link is halted in the presence of hypertension or atherosclerosis as assessed by ABI. Alternatively, the raised HGF serum level may be an epiphenomenon of atherosclerosis.

Key words: ankle-brachial index, atherosclerosis, cognition, hepatocyte growth factor, hypertension

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Conflict of interest

The Authors declare no conflict of interest

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INTRODUCTION

Hepatocyte growth factor (HGF) is a heparin-binding polypeptide (728 amino acids) that regulates the growth, migration, and morphogenesis of various cells ¹. An increased serum level of HGF has been reported in patients with hypertension, peripheral arteriosclerosis, carotid atherosclerosis, and coronary artery disease ^{1,2}.

HGF has been shown to have an anti-apoptotic action on the endothelium ³, it has been detected in atherosclerotic plaques ⁴, and it shows a positive association with atherosclerosis ⁵.

Atherosclerotic vascular disease affects large- and medium-sized arteries of most circulatory beds and it is the leading cause of death and disability in developed countries. Lower-extremity atherosclerosis, peripheral arterial disease (PAD), is a significant public health problem ⁶.

There is a controversy about the association between HGF serum level and cognition. Although some consider HGF as a new targeted therapy in dementia ⁷, others found that it is associated with cognitive dysfunction ⁸, with reports about increased HGF levels in the cerebrospinal fluid (CSF) of patients with AD ⁹. Similarly, others found that HGF serum level is associated with the presence of cardiovascular disease (CVD) risk factors ¹⁰. Recently, literature has increased interest in evaluating the link between dementia and serum HGF ¹¹. ZhuY et al found that magnetic resonance imaging (MRI) markers of small vessel disease (SVD) rather than large vessel disease were associated with higher serum HGF, among those with cognitive impairment not demented or Alzheimer's disease ¹¹.

As previous literature reported the presence of SVD in normal elderly ¹² and diabetics ¹³, along with the supposed cross talk between large and small arteries in hypertensives ¹⁴, the current work aimed to explore the link between HGF serum level and cognition in hypertensive elderly with abnormal ankle-brachial index (ABI), as a known marker of atherosclerosis ^{15,16}, and hypertensive elderly with normal ABI versus controls (normotensives with normal ABI), among non-diabetic subjects.

As atherosclerosis, which is of a major link to hypertension ¹⁷, is linked to vascular dementia ⁸, we hypothesized that hypertension or atherosclerosis with hypertension could alter the relation between HGF serum level and cognitive function.

MATERIALS AND METHODS

A case-control study was conducted among elderly subjects, aged ≥ 60 years ^{18,19}, attending outpatient primary care geriatric clinic. Ninety eligible elderly patients were consecutively included and were subdivided into 3 groups. Group A included 30 normotensive subjects with normal ABI as controls, group B included 30 hypertensive subjects with normal ABI as cases and group C included 30 hypertensive subjects with abnormal ABI as cases. The data were collected from January 2017 to June 2017.

Those with diabetes mellitus, severe liver or renal dysfunction or cancer were excluded from the study because serum levels of HGF are well known to be influenced by these diseases ^{20,21}.

Subjects were excluded if they had severe sensory or cognitive impairment which could interfere with the assessment or refused to participate.

The minimum sample size was based upon the odd ratio for the association between ABI and cognitive test performance in elderly ²². Using Epi-info program, version 3.5.1, power 1- β 80%, and confidence interval of 95%, the minimum number was 16 for cases and 16 for controls.

COGNITIVE FUNCTION

It was assessed by The Rowland Universal Dementia Assessment Scale (RUDAS). The RUDAS is a cognitive assessment tool that was created for culturally and linguistically diverse populations. The RUDAS is a 6-item questionnaire that assesses multiple cognitive domains and can be administered in less than 10 minutes. In the original validation study of RUDAS, both the interrater and test-retest reliabilities of the test were very high. Compared with the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), the RUDAS was found to have a sensitivity of 89% and a specificity of 98%. Performance on the RUDAS was not affected by years of education or the preferred language ²³.

DIAGNOSIS OF HYPERTENSION

Blood pressure was measured using a standard mercury sphygmomanometer in the right arm, in supine position after rest for 5 minutes. Systolic and diastolic blood pressure was recorded as the mean of two measurements. The diagnosis of hypertension was based upon known history of hypertension diagnosis and its treatment or it was based upon the cutoff values stated by the National heart foundation of Australia, systolic ≥ 140 mmHg and/ or diastolic ≥ 90 mmHg, on two separate occasions, at least one week apart ²⁴.

MEASUREMENT OF ANKLE-BRACHIAL INDEX

The ABI is an objective non-invasive reproducible measure that reflects PAD severity ¹⁵, and ABI is considered as a marker of atherosclerosis in the Cardiovascular Health Study, Cardiovascular Heart Study Collaborative Research Group ¹⁶.

ABI was assessed by hand-held vascular Doppler, BT-200, HI-dop. Steps were applied according to known references, with abnormal ABI < 0.9 ^{11,25}.

LABORATORY DATA

SAMPLING

Five milliliters of fasting venous blood were collected under complete aseptic precautions in plain test tubes.

The serum was separated by centrifugation (1000x g for 15 minutes) and was divided into two aliquots. One was designated for the immediate assay of fasting lipid profile. The other aliquot was stored at -20°C for subsequent assay of HGF. Hemolysed samples were discarded. Repeated freezing and thawing were avoided.

ANALYTICAL METHODS

Serum fasting lipid profile [total cholesterol, triglycerides (TG) and high-density lipoprotein- cholesterol (HDL-C)] was measured using Synchron CX-9 autoanalyser (Beckman Instruments Inc.; Scientific Instruments Division, Fillerton, CA 92634, 3100, USA). Low density lipoprotein- cholesterol (LDL-C) value was calculated according to Friedewald equation. HGF assay was done using the commercially available enzyme-linked immunosorbent assay (ELISA) kit, supplied by Elabscience Company (Building 4, Room 401, Guandong Science and Technology Industry Park, Wuhan, P.R.C.).

STATISTICAL METHOD

SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Qualitative data were expressed in the form of number and percentage and were compared using Chi-Square test. Quantitative data were expressed in the form of mean \pm SD for

parametric data or median and interquartile range for non-parametric data and were compared using ANOVA test (with least significant difference as post-hoc test), after log transformation of non-parametric data.

Distribution normality was assessed using a z test for skewness²⁶.

Generalized linear model was used to study the significance of serum HGF level as a predictor of cognitive function, linear distributional assumption was used, in each group, after adjustment for the possible confounding variables.

RESULTS

Using ANOVA, there was no significant difference between the 3 groups in age ($p = 0.98$).

Serum HGF concentration was higher in group C (hypertensives with abnormal ABI) compared with group A (controls) or B (hypertensives with normal ABI) ($p < 0.001$ and 0.025 consecutively), and serum HGF concentration was higher in group B than group A ($p = 0.028$) (Tab. I).

Group C had worse RUDAS score than controls ($P = 0.01$), with no significant difference between group B and C in RUDAS score ($p = 0.22$) (Tab. I).

Table I. Comparing characteristic data between the 3 groups¹⁸.

	Group A (controls)	Group B (hypertensives with normal ABI)	Group C (hypertensives with abnormal ABI)	P-value	P1	P2	P3
Age (years)	69.20 \pm 10.31	69.23 \pm 7.04	69.60 \pm 7.28	0.98			
Males (n, %)	24 (80%)	14 (46.7%)	16 (53.3%)	0.02			
Education years*	0 (0-6)	0 (0-5.5)	0 (0-6)	0.84			
Smoking Index*	130 (0-142.5)	0 (0-60)	0 (0-137.5)	0.31			
IHD	7 (25.0%)	9 (30.0%)	25 (83.3%)	< 0.001			
Stroke	1 (3.6%)	2 (6.7%)	3 (10.0%)	0.62			
SBP (mmhg)	123.3 \pm 11.24	134.7 \pm 16.3	132.7 \pm 12.9	0.004	0.002	0.01	0.57
DBP (mmhg)	77.67 \pm 6.79	82.33 \pm 9.32	81 \pm 8.9	0.092			
BMI*	24 (21.7-26.75)	29.4 (25.4-32)	26.6 (23.6-36.7)	< 0.001	0.001	0.001	0.99
WC (cm)	96 \pm 17.18	112.6 \pm 3.8	109.2 \pm 21.19	0.011	0.006	0.02	0.59
GDS-15 score*	2 (1.75-3)	2 (0-3)	2 (1-4.75)	0.14			
T chol. (mg/dl)	137 \pm 47.7	186.6 \pm 59.7	164.1 \pm 50.8	0.003	0.001	0.051	0.11
TG (mg/dl)	114.9 \pm 50.2	146.9 \pm 54.5	149.1 \pm 57.9	0.03	0.033	0.02	0.88
LDL-C (mg/dl)	87.1 \pm 36.8	122 \pm 36.6	104.9 \pm 39.9	0.004	0.001	0.08	0.09
HDL-C (mg/dl)	37.8 \pm 12.43	43.5 \pm 17.37	37.79 \pm 11.02	0.22			
ABI*	0.93 (0.91-0.97)	0.93 (0.92-0.93)	0.6 (0.5-0.7)	< 0.001	0.82	< 0.001	< 0.001
RUDAS score	27.13 \pm 2.78	26.13 \pm 3.05	25.2 \pm 3.30	0.049	0.21	0.01	0.22
HGF (pg/ml)*	170 (100-350)	345 (130-838)	795 (250-1343.8)	< 0.001	0.028	< 0.001	0.025

ABI: Ankle-Brachial Index; BMI: Body Mass Index; DBP: Diastolic Blood Pressure; GDS-15: Geriatric Depression Scale-15 items; HDL-C: High Density Lipoprotein-cholesterol; HGF: Hepatocyte Growth Factor; IHD: ischemic heart disease; LDL-C: Low Density Lipoprotein-cholesterol; RUDAS: Rowland Universal Dementia Assessment SCALE, SBP: Systolic Blood Pressure; T chol.: Total cholesterol; TG: Triglycerides; WC: waist circumference; *: data were expressed as median (interquartile range) for non-parametric quantitative data; P1, P2 and P3: P values for post-hoc analysis; P1: Group A vs Group B; P2: Group A vs Group C; P3: Group B vs Group C.

By Pearson correlation, serum HGF concentration was positively correlated with RUDAS score only in group A ($p = 0.035$, $r = 0.4$). HGF concentration was negatively correlated with ABI, in group C ($p = 0.007$, $r = -0.49$), and it was positively correlated with systolic blood pressure (SBP), in group B ($p = 0.045$, $r = 0.37$). In group C, HGF was positively correlated with SBP, after adjustment for ABI. Serum HGF concentration was not correlated with lipid profile.

Generalized linear model, in each group, revealed the followings; in group A, serum HGF concentration was positively associated with RUDAS score ($p < 0.001$). In group B, serum HGF concentration was positively associated with RUDAS score only after further adjustment for SBP ($p = 0.024$). In group C, serum HGF concentration was positively associated with RUDAS score only after adjustment for ABI or ABI and SBP ($p = 0.031$ and 0.05 consecutively) (Tab. II).

After further adjustment for lipid profile, the significant association between HGF and RUDAS score was found only in controls ($p = 0.006$, OR = 1.004 and CI = 1.001-1.006).

Furthermore, adjustment for gender, body mass index, ischemic heart disease and lipids doesn't affect the significant association between HGF and RUDAS score

in controls ($p = 0.017$, OR = 1.004 and CI = 1.001-1.006).

DISCUSSION

The current data explored that HGF was higher in hypertensives with/or without abnormal ABI than controls, and hypertensives with abnormal ABI had higher HGF than hypertensives with normal ABI. Therefore, HGF might mediate the pathology of hypertension \pm atherosclerosis. Alternatively, the raised HGF serum level may be an epiphenomenon of hypertension \pm atherosclerosis.

This is in accordance with Yoshitomi et al.²⁷ who found that patients with PAD showed higher serum HGF concentrations than controls.

Similarly, Nakamura et al.²⁸ explored that serum HGF concentration in hypertensive subjects without any complication was higher than normotensive subjects ($p < 0.001$). Furthermore, serum HGF concentration in hypertensive patients with complications was significantly higher than those without complication or normotensive subjects.

Recently, Bell et al.²⁹ proved the positive association

Table II. Predictors/associates of RUDAS score, in each group.

Groups	Parameter	B	Sig.	O.R.	95% Confidence Interval for O.R.	
					Lower	Upper
Controls						
• Step 1:	HGF	3.172	< 0.001	23.846	8.276	68.712
• Step 2:	HGF	3.537	< 0.001	34.370	10.409	113.484
	ABI	14.494	0.017	1.971E6	12.747	3.046E11
• Step 3:	HGF	4.281	< 0.001	72.288	21.288	245.475
	ABI	7.880	0.204			
	SBP	0.110	< 0.001	1.116	1.074	1.160
Hypertensive with normal ABI						
• Step 1:	HGF	0.512	0.246			
• Step 2:	HGF	0.564	0.206			
	ABI	7.405	0.411			
• Step 3:	HGF	1.081	0.024	2.948	1.155	7.526
	ABI	6.561	0.467			
	SBP	-0.037	0.003	0.964	0.941	0.988
Hypertensive with abnormal ABI						
• Step 1:	HGF	0.099	0.782	1.104	0.550	2.216
	HGF	0.878	0.031	2.407	1.084	5.342
• Step 2:	ABI	6.694	< 0.001	807.519	29.081	22423.449
	HGF	0.789	0.05	2.201	0.989	4.900
• Step 3:	ABI	5.936	0.001	378.581	12.984	11038.819
	SBP	-0.038	0.009	0.963	0.935	0.991

ABI: Ankle-Brachial Index; HGF: Hepatocyte Growth Factor; RUDAS: Rowland Universal Dementia Assessment Scale; SBP: Systolic Blood Pressure; step 1: HGF was used in regression alone; step 2: adjusted regression for ABI; step 3: adjusted regression for ABI and systolic blood pressure.

between higher circulating HGF levels at baseline and the progression of atherosclerosis, as defined by coronary artery calcium and carotid plaques, among participants aged 45-84 years. Additionally, Decker et al.³⁰ reported positive relation between changes in HGF levels and clinical coronary heart disease; rather than subclinical atherosclerosis, as defined by coronary artery calcium.

The positive association between SBP and HGF serum level, in the current work, was in accordance with Nakamura et al.²⁸.

The current results explored that serum HGF concentration was negatively associated with ABI, as a marker of atherosclerosis^{15,16}. This might be explained by Taher et al.⁴ who declared that the migration of vascular smooth muscle cells (VSMCs) to the intima is the main event in neo-intima formation and atherogenesis. They found that Met, the receptor for HGF, is expressed on VSMCs derived from the intima of atherosclerotic plaques. Furthermore, HGF promoted VSMC migration across fibronectin-coated filters. Their findings suggested a role for the HGF in the pathogenesis of atherosclerosis and restenosis.

Although some previous evidence found a possible role for serum HGF concentration for the treatment of PAD, through angiogenesis and improved necrosis in rat ischemic limbs³¹, these positive results were not the same for humans in two multicenter, double blind, placebo controlled clinical trials by Powell et al.³² and Shigematsu et al.³³.

In the current study, serum HGF concentration was positively associated with cognitive performance, in controls. These data are supported by previous experimental studies which revealed improvement in neurodegenerative diseases by HGF^{34,35}.

The beneficial effect of HGF could be explained by Takeuchi et al who found that injection of A-beta peptide decreases the blood-vessel density which improved after HGF expression³⁶. A decrease in cerebral blood flow is observed in Alzheimer disease patients³⁷.

These findings are also attributed to the anti-apoptotic effect of HGF on endothelium via Bcl-2 induction, Bcl-2 is an inhibitor of apoptosis³.

On the other hand, previous studies found increased HGF levels in the CSF of patients with AD⁹, however other cardiovascular risk factors were not considered. Recently, ZhuY et al. linked MRI markers of SVD, rather than large vessel disease, to higher serum HGF levels, among those with cognitive impairment not demented or Alzheimer's disease subjects, even after controlling for cardiovascular risk factors¹¹. ZhuY et al. excluded vascular dementia and stated that the precise mechanism for small rather than large vessel disease is unclear.

Hypertensives with abnormal ABI had worse RUDAS score than controls, and in cases, serum HGF concentration was positively associated with RUDAS score, only after further adjustment for SBP and ABI. These findings suggest the negative impact of hypertension and atherosclerosis on the positive association between HGF serum concentration and cognitive function. After further adjustment for lipid profile, the significant association between HGF and RUDAS score was found only in controls. Although of the presence of traditional knowledge about the deleterious effect of lipids upon cognition³⁸, this is not a well established risk in recent intervention studies³⁹, even some studies linked higher levels of cholesterol, triglyceride and LDL in older adults to a lower risk for vascular dementia and better cognitive performance⁴⁰⁻⁴².

CONCLUSIONS

The potential beneficial link between serum HGF concentration and cognitive function could be met in normotensive subjects with normal ABI. However, this link is halted in the presence of hypertension or atherosclerosis, as assessed by ABI, which was negatively associated with serum HGF concentration. Alternatively, the raised HGF serum level may be an epiphenomenon of atherosclerosis.

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