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Chitotriosidase as a novel biomarker of early atherosclerosis in hemodialysis patients

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Abstract

Introduction: Increasing evidence suggests that inflammation and increased macrophage activity have a central role in pathogenesis of atherosclerosis. It is shown that chitotriosidase (CHIT-1) is a marker of macrophage activity in atherosclerotic plaque, and is found associated with severity of atherosclerotic lesion. There is no data about CHIT-1 activity of hemodialysis patients in the literature. Thus, we hypothesized that in hemodialysis patients, CHIT-1 levels might be a novel biomarker in early atherosclerosis. **Methods:** Forty-five hemodialysis patients were included in the study (age: 61.93 ± 13.34). Intima media thickness (IMT) was evaluated with high-resolution B-mode ultrasonography. Biomarker levels were measured in serum of patients. **Findings:** We found positive correlation among IMT, age (*R*: 0.426, *P*: 0.004) and, CHIT-1 value (*R*: 0.462, *P*: 0.001) in spearman correlation analysis. When age, CRP, creatinine, *P*, Alb, CHIT-1 were chosen as measures that can effect IMT in multiple regression model, IMT level was related with CHIT-1 (Beta: 0,396, *P*: 0.012) and age (Beta: 0,313 *P*: 0,048) independently. **Discussion:** In conclusion, this is the first report showing that serum CHIT-1 level was related independently with carotid IMT in hemodialysis patients. This biomarker might have an unknown role in the development of atherosclerosis during uremia.

Key words: Chitotriosidase (CHIT-1), atherosclerosis, intima media thickness, hemodialysis

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INTRODUCTION

Carotid artery intima media thickness (IMT) is an independent predictor of coronary artery disease.[1] IMT was found related with atherosclerotic cardiovascular disease in many conditions such as hypertension, rheumatoid arthritis, and chronic kidney failure (CKF).[2–4] Increased carotid intima thickness is frequently seen in moderate and advanced kidney disease and related with high cardiovascular mortality and morbidity in this population.[5] These high mortality and morbidity rates cannot be explained with

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traditional cardiovascular risk factors alone; thus non-traditional risk factors such as inflammation are considered especially in patients with CKF. Increasing evidence shows that inflammation and increased macrophage activity have a central role in pathogenesis of atherosclerosis.

Chitotriosidase (CHIT-1), which is a member of chitinase family, attracted notice in last decade and is shown to be a marker of macrophage activity in atherosclerotic plaque.[6] In patients with atherothrombotic stroke and ischemic heart disease, CHIT-1 activity was found to be higher than healthy controls.[7] CHIT-1 activity was found related with severity of atherosclerotic lesion.[8,9] Thus, we hypothesized that CHIT-1 might be a novel biomarker of early atherosclerosis in hemodialysis patients. In addition to this, there is no data about CHIT-1 activity of hemodialysis patients in the literature. In this study, we aimed to evaluate the relation between serum CHIT-1 level and IMT, which is a marker of early atherosclerosis, in hemodialysis patients.

MATERIALS AND METHODS

Patients

Patients aged between 18 and 80, who had received hemodialysis treatment for at least 1 year in Hemodialysis Unite in Ahi Evran University Faculty of Medicine Hospital were included in this study. Patients with active infection, malignity, documented cardiovascular disease, immunosuppression were excluded from the study. After exclusion, 45 hemodialysis patients were included in the study. Mean age of patients in this study was 61.93 ± 13.34. Clinic characteristics of study group are shown in Table 1. Systolic and diastolic blood pressures of patients were measured with ERKA sphygmomanometer after 15 minutes of rest. Hypertension was defined as use of antihypertensive drug use or systolic/diastolic blood pressure higher than 140/90 mmHg in repeated measurements, respectively. All procedures of the study were done properly in the context of national and institutional ethical guidelines. Study was approved by the Clinical Research Ethical Committee of Dumlupınar University.

Laboratory measurements

Hemogram and plasma triglyceride, HDL, LDL, Ca, P, CRP, Na, K, ferritin, uric acid, and parathyroid hormone levels were measured in patients after one night of fasting. Complete blood count measurement was completed with flow cytometry method; fasting blood glucose, creatinine, albumin, serum lipid measurements were completed with enzymatic colorimetric method; Ferritin and C-reactive protein (CRP) measurements were completed with immunoturbidimetric method; and sodium, potassium, and chloride measurements were completed with ion selective electrode method. Sysmex XT 2000I device was used for complete blood count and all other biochemical parameters were measured with Modular P, Roche/Hitachi device.

CHIT-1 levels were determined by sandwich ELISA kit. CHIT-1 concentrations were assayed according to the CHIT-1 ELISA protocol of manufacturer's instruction (Human Chitinase 1 (Chitotriosidase) ELISA Kit (Cusabio Biotech Co., Ltd. Wuhan, China)). Briefly, 100 μL of standards and samples were added to 96-microwell plate wells and incubated 2 hours at 37°C. After incubation, liquid of each well was aspirated and 100 μL Biotin Antibody was added to each well. The plate was incubated for 1 hour at 37C. After incubation, each well was washed

Table 1 Characteristics of patients in hemodialysis group

Characteristic	Hemodialysis (*n*: 45)
Age (year; mean ± SD)	61.93 ± 13.34
Male gender (*n*)	19
Systolic blood press. (mmHg, mean ± SD)	120.75 ± 18.86
Diastolic blood press. (mmHg, mean ± SD)	77.87 ± 14.00
Cause of CKD	
Hypertension (*n*)	11
mellitus (*n*)	14
Urolithiasis (*n*)	1
Unknown (*n*)	19
Medications	
Angiotensin converting enzyme inh. (*n*)	4
Beta-blocker (*n*)	8
Statins (*n*)	0
Erythropoietins (*n*)	33
Phosphorus binders (*n*)	37
Vitamin D (*n*)	18
UF in one session (cc)	3100.00 ± 570.08
Hemodialysis duration (Month, mean ± SD)	80.00 ± 50.32
CKD duration (Month, mean ± SD)	91.46 ± 50.34

CKD = chronic kidney disease; *N* = number of patients; SD = standard deviation; UF = ultrafilitration.

Table 2 Laboratory and vascular evaluation in hemodialysis group

	Hemodialysis (*n*: 45) mean ± SD
Leukocyte (10^3/uL)	7.073 ± 2.11
Hemoglobin (g/dL)	120.75 ± 18.86
Platelets (10^3/uL)	203.11 ± 82.51
Serum creatinine (mg/dL)	8.46 ± 2.28
Triglyceride (mg/dL)	200.72 ± 94.79
LDL-cholesterol (mg/dL)	98.95 ± 33.03
HDL-cholesterol (mg/dL)	35.07 ± 8.7
Total cholesterol (mg/dL)	166.66 ± 42.96
Phosphate (mg/dL)	4.80 ± 1.22
Na (mmol/L)	137.75 ± 2.55
K (mmol/L)	5.52 ± 0.78
Uric acid (mg/dL)	6.54 ± 1.01
Ferritin (mg/dL)	799.16 ± 262.08
Albumin (g/dL)	3.82 ± 0.29
CRP (mg/dL)	2.03 ± 3.04
Parathyroid hormone (pg/mL)	325.15 ± 361.73
KT/V	1.52 ± 0.23
IMT (mm)	0.92 ± 0.19
Chitotriosidase (pg/mL)	2211.63 ± 800.06

CRP = C reactive protein; HDL = high density lipoprotein; IMT = intima media thickness; LDL = low density lipoprotein; SD = standard deviation.

three times with 200 μL wash buffer. Then, 100 μL HRP-avidin was added to each well and the plate was incubated at 37°C for 1 hour. After incubation, each well was washed five times with 200 μL wash buffer. About 90 μL of TMB substrate solution was added to each well and the plate was incubated in the dark at 37°C for 20 minutes. Then, 50 μL of stop solution was added to each well and the plate was used to measure the absorbance at 450 nm with a Snergy HT plate reader (BioTek Instruments, Winooski, Vermont, USA). From the standard curve, the CHIT-1 levels in each test sample were quantitated.

Measurement of carotid intima-media thickness

IMT of carotid artery was measured as a marker of subclinical atherosclerosis. Carotid artery ultrasonographic findings of patients were done by only one radiologist in Ahi Evran University Faculty of Medicine, Department of Radiology. Toshiba Aplio MX, Japan high-resolution ultrasonography with 4–11 mHz linear probe was used. Measurement of area between two echogenic lines of interfaces of intima-lumen and media-adventitia was defined as IMT. Measurement data was acquired with optimal depth, focus, frequency, and gain adjustments. Three different measurements of left main carotid artery (from 1 to 2 cm proximal of bulbus) were performed when patient was in supine position and turned their head to left.[10] Average of measurements was defined as IMT value. We didn't perform any measurement if there was atheroma plaque in the location. Imaging was done in axial and longitudinal planes; posterior wall was used in measurements.

Statistical analysis

Non-normally distributed variables were expressed as median (range) and normally distributed variables were as mean ± SD as appropriate. A P value <0.05 was considered to be statistically significant. Between-group comparisons were performed for nominal variables using the Chi-square test. Spearman's rank correlation was used to determine correlations with continuous variables. Stepwise multivariate regression analysis was used to assess the predictors for IMT levels. All the statistical analyses were performed by using SPSS 19.0 (SPSS Inc., Chicago, IL) statistical package.

RESULTS

Characteristics of patients in this study are shown in Tables 1 and 2. Conventional risk factors of patients in this study are shown in Table 3. We identified a positive correlation among IMT, CHIT-1 level ($r = 0.462$, $P < 0.001$), and age ($r = 0.426$, $P = 0.004$) and in spearman correlation analysis (Figures 1 and 2).

Table 3 Multivariable regression analysis of predictors of carotid intima media thickness

	N (45) /%
Age Men >45 Women >55	37/82.2
Male gender	19/42
Hypertension	4/8.8
LDL >130, T. Coll >200	10/22.2
HDL <40	32/71.1
Diabetes mellitus	15/33.3
Smoking	4/8.8
Family history	6/13.3

CRP = C reactive protein; HDL = high density lipoprotein; T. Coll. = total cholesterol;.

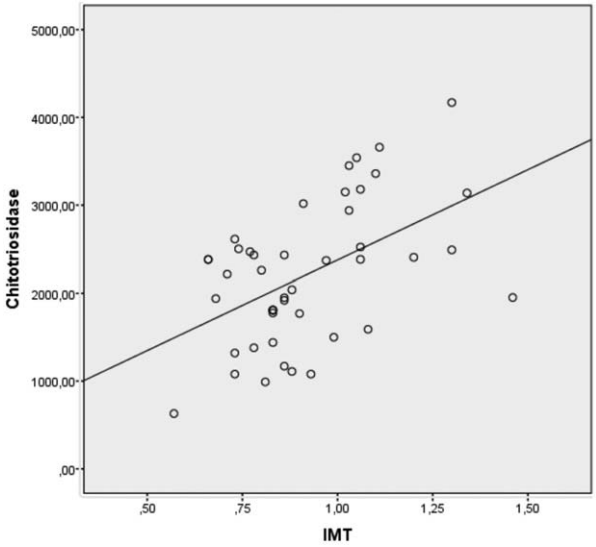

Figure 1 The relationship between CHIT-1 and carotid IMT. $r = 0.462$, $P < 0.001$. IMT: intima media thickness.

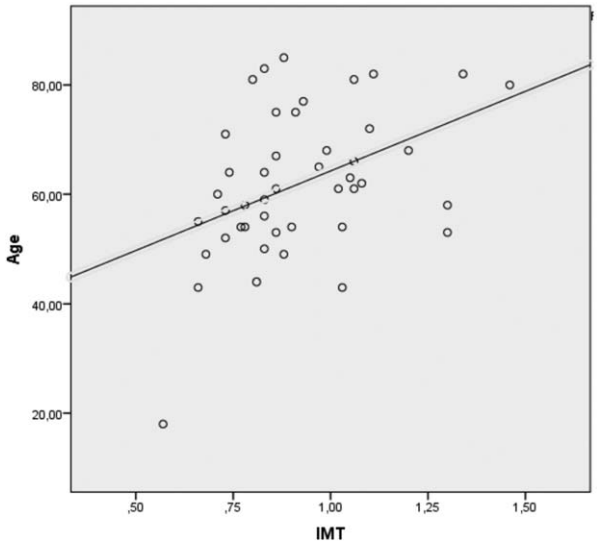

Figure 2 The relationship between age and carotid IMT. $r = 0.426$, $p = 0.004$. IMT: Intima media thickness

We used multiple regression models to find whether CHIT-1 was an independent marker of IMT. When we chose age, CRP, creatinine, *P*, Alb as measures that might affect IMT, IMT value was found independently associated with CHIT-1 level (beta = 0.396, $P = 0.012$) and age (beta = 0.313, *P*, 0.048) (Table 4).

DISCUSSION

In our study we showed an independent relation between serum CHIT-1 level and, carotid IMT which is a marker of early atherosclerosis, for the first time.

CKF is an independent risk factor for atherosclerotic cardiovascular diseases and related with higher cardiovascular mortality and morbidity compared to normal population.[11,12] Traditional risk factors cannot explain significant increase of atherosclerosis incidence in kidney failure patients alone and non-traditional risk factors such as inflammation becomes important. Recent laboratory and clinical studies[14,15] support Roos' findings[13] that suggest macrophages have a central role in atherosclerosis progress which is an inflammatory process. Also macrophages are shown to be a marker of atherosclerotic plaque formation in all phases of atherogenesis.[16]

Our main finding in this study is the independent relation between CHIT-1 and carotid IMT for the first time. This might be caused by increased macrophage activity of atherosclerosis pathogenesis in uremic patients. Pathomechanism of chitotriosidas in macrophages is shown in Figure 3.[17,18] Numerous markers of systemic inflammation and macrophage activation were found associated with cardiovascular events.[19] Serum CHIT-1 level was shown to be a marker of macrophage activity in atherosclerotic

Table 4 Multivariable regression analysis of predictors of carotid intima media thickness

			%95 Confidence interval	
Dependent value: IMT	Beta	*P*	Lower limit	Upper limit
Chitotriosidase (pg/mL)	0.396	0.012*	0.001	0.005
Age (year)	0.313	0.048*	0	0.009
CRP (mg/dL)	−0.02	0.918	−0.03	0.026
Serum creatinine (mg/dL)	−0.06	0.705	−0.03	0.022
Phosphate (mg/dL)	−0.11	0.474	−0.07	0.031
Albumin (g/dL)	0.231	0.181	−0.08	0.392
Model $R^2 = 0.413$; Model $P = 0.002$				

*<0.005.

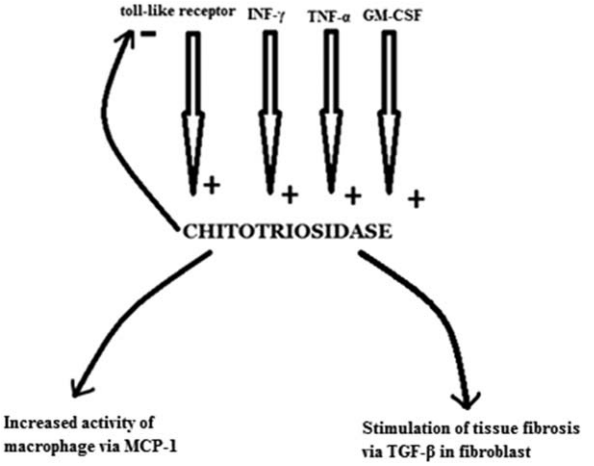

Figure 3 Pathomechanism of chitotriosidas in macrophages expression of chitotriosidas increases based on (TLR), (IFN)-γ, (TNF)-α, GM-CSF. Increased CHIT-1 is down-regulated over TLR. Increase of chit activity increases macrophage activity over via MCP-1, while stimulating tissue fibrosis by increasing fibroblast activity via TBF-beta. Pathomechanism of chitotriosidas in macrophages. INF-γ: interferon-gamma, TGF-β: transforming growth factor-beta, TNF-α: tumor necrosis factor-alpha. GM-CSF: granulocyte macrophage colony-stimulating factor, MCP-1: monocyte chemotactic protein, TLR: Toll-like receptor

plaque.[6] CHIT-1 activity was found 55 times higher in atherosclerotic tissue extracts and high serum CHIT-1 activity was related with width of atherosclerotic lesion.[20] All these findings point out a strong correlation between CHIT-1 activity and lipid-loaded macrophages in atherosclerotic vein wall.

In line with our findings, Safarinejad and Safarinejad showed that plasma CHIT-1 activity was increased in normolipidemic atherosclerotic patients and found a significant correlation between plasma CHIT-1 activity and IMT.[21] CHIT-1 activity was higher in patients with atherosclerotic stroke and ischemic heart disease than healthy controls.[8] Canudas et al.[9] found CHIT-1 activity was associated with severity of atherosclerotic lesion. CHIT-1 level was shown to be more effective in predicting atherosclerosis in dyslipidemic children compared to hs-CRP, IL-6, and TNF-α.[22] Also serum CHIT-1 activity was shown to be a strong marker of coronary artery disease.[23] Artieda et al. showed that CHIT-1 level was increased in atherothrombotic groups of 153 patients with atherothrombotic stroke and 24 patients with unstable angina pectoris.[8] Differently from our findings, Arterida et al. identified a correlation between serum CHIT-1 level and age. In our study, we identified a relation between age and CHIT-1 level close to the margin of statistical significance; however we couldn't identify statistical significance ($r = 0.261$, $P = 0.82$). This might be due to lower population of our study group compared to study group of Arterida et al., or whether CHIT-1 activity is effected by ethnicity or different genotype.[24,25]

Kologlu et al.[22] identified a relation between serum CHIT-1 level and lipid levels in dyslipidemic children. However we couldn't identify any relation between CHIT-1 level and serum lipid levels. Also Karadağ et al. couldn't identify any relation between lipid levels and CHIT-1 level similar to our findings.[23] Canusa et al. couldn't identify any relation between serum lipid levels and macrophage CHIT-1 expression.[9] Absence of relation between lipid levels and serum CHIT-1 level in our study might be due to normolipidemic nature of patients in our group.

Limitations of this study are low population number of patient group and absence of control and CKF group. Yet independent relation with intima media remains important. Such as low-mediated dilatation and pulse wave velocity should be done to show the association between vascular status and CHIT. However our study is a preliminary study, thus we plan to analyze these parameters, which show vascular status in more detail, in our next study.

In conclusion, this is the first report showing that serum CHIT-1 level was related independently with carotid IMT in hemodialysis patients. This biomarker might have an unknown role in development of atherosclerosis during uremia.

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REFERENCES

1 Jadhav UM, Kadam NN. Carotid intima-media thickness as an independent predictor of coronary artery disease. *Indian Heart J.* 2001; **53**:458–462.
2 Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: The Rotterdam Study. *Circulation.* 1997; **96**:1432–1437.
3 Izzo R, Stabile E, Esposito G, et al. Development of new atherosclerotic plaque in hypertensive patients: An observational registry study from the Campania-Salute network. *J Hypertens.* 2015; **33**:2471–2476.

4 Kisiel B, Kruszewski R, Juszkiewicz A, Kłos K, Tłustochowicz M, Tłustochowicz W. Prevalence of Atherosclerosis in diabetic and non-diabetic patients with rheumatoid arthritis. *Pak J Med Sci.* 2015; **31**:1078–1083.
5 Adeseun GA, Xie D,Wang X, et al. Carotid plaque, carotid intima-media thickness, and coronary calcification equally discriminate prevalent cardiovascular disease in kidney disease. *Am J Nephrol.* 2012; **36**:342–347.
6 Moreno, PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration in acute coronary syndromes: Implications for plaque rupture. *Circulation.* 1994; **90**:775–778.
7 Brinkman J, Wijburg FA, Hollak CE, et al. Plasma chitotriosidase and CCL18: Early biochemical surrogate markers in type B Niemann-Pick disease. *J Inherit Metab Dis.* 2005; **28**:13–20.
8 Artieda M, Cenarro A, Ganan A, et al. Serum chitotriosidase activity is increased in subjects with atherosclerosis disease. *Arterioscler Thromb Vasc Biol.* 2003; **23**:1645–1652.
9 Canudas J, Cenarro A, Civeira F, et al. Chitotriosidase genotype and serum activity in subjects with combined hyperlipidemia: Effect of the lipid-lowering agents, atorvastatin and bezafibrate. *Metabolism.* 2001; **50**:447–450.
10 Sidhu PS, Desai SR. A simple and reproducible method for assessing intimal-medial thickness of the common carotid artery. *Br J Radiol*. 1997; **70**:85–89.
11 Lai S, Dimko M, Galani A, et al. Early markers of cardiovascular risk in chronic kidney disease. *Ren Fail.* 2015; **37**:254–261.
12 Astor BC, Coresh J, Heiss G, Pettitt D, Sarnak MJ. Kidney function and anemia as risk factors for coronary heart disease and mortality: The Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J.* 2006; **151**:492–500.
13 Ross R. Atherosclerosis: An inflammatory disease. *N Engl J Med.* 1999; **340**:115–126.
14 Young JL, Libby P, Schonbeck U. Cytokines in the pathogenesis of atherosclerosis. *Thromb Haemost.* 2002; **88**:554–567.
15 Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. *J Intern Med* 2002; **252**: 283–294.
16 Stary HC, Chandler AB, Glagov S, et al. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis: A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis. American Heart Association. *Circulation.* 1994; **89**: 2462–2478.
17 Malaguarnera L, Musumeci M, Di Rosa M, Scuto A, Musumeci S. Interferon-gamma, tumor necrosis factor-alpha, and lipopolysaccharide promote chitotriosidase gene expression in human macrophages. *J Clin Lab Anal.* 2005; **19**:128–132.
18 Van Eijk M, Scheij SS, van Roomen CP, Speijer D, Boot RG, Aerts JM. TLR- and NOD2-dependent regulation of human phagocytespecific chitotriosidase. *FEBS Lett.* 2007; **581**:5389–5395
19 Hansson GK. Inflammation, atherosclerosis and coronary artery disease. *N Engl J Med.* 2005; **352**:1685–1695.
20 Boot RG, Van Achterberg TA, Van Aken BE, et al. Strong induction of members of the chitinase family of proteins in atherosclerosis: Chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. *Arterioscler Thromb Vasc Biol.* 1999; **19**:687–694.
21 Safarinejad MR, Safarinejad S. Plasma chitotriosidase activity and arteriogenic erectile dysfunction: Association with the presence, severity, and duration. *J Sex Med.* 2010; DOI:10.1111/j.1743-6109.2009.01673.x
22 Kologlu T, Ucar SK, Levent E, Akcay YD, Coker M, Sozmen EY. Chitotriosidase as a possible marker of clinically evidenced atherosclerosis in dyslipidemic children. *J Pediatr Endocrinol Metab.* 2014; **27**:701–708.
23 Karadag B, Kucur M, Isman FK, Hacibekiroglu M, Vural VA. Serum chitotriosidase activity in patients with coronary artery disease. *Circ J.* 2008; **72**:71–75.
24 Hollak CE, Van Weely S, Van Oers MH, Aerts JM. Marked elevation of plasma chitotriosidase activity: A novel hallmark of Gaucher disease. *J Clin Invest.* 1994; **93**:1288–1292.
25 Eiberg H, den Tandt WR. Assignment of human plasma methylumbelliferyl-tetra-*N*-acetylchitotetraoside or chitinase to chromosome 19 by a linkage study. *Hum Genet.* 1997; **101**:205–207.