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Plasma Concentration of Osteopontin as A Predictor of Vascular Calcification in Patients with Diabetic Nephropathy

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ABSTRACT

Background: Vascular calcification (VC) is a common health problem in patients with diabetic nephropathy (DN). Early recognition of vascular calcification has a great effect on the outcome.

Objective: We aimed to assess the efficacy of serum osteopontin (OPN) in the early prediction of VC in patients with diabetic nephropathy.

Patients and Methods: A total of 70 patients with type 2 diabetes mellitus were divided into equal two groups. Group (A): included 35 patients with diabetic nephropathy with chronic kidney disease (CKD) stage I-IV and group (B): included 35 patients with DN with CKD stage V on hemodialysis. Another 30 healthy subjects were recruited as a control group. Carotid Doppler and echocardiography were done in patients' groups only while serum OPN was measured in all groups.

Results: Osteopontin was found to be higher in group B in comparison to other groups and also higher in group A in comparison control group. A total of 31 (44.3%) patients had VC. Patients with VC had higher OPN in comparison to those without VC. Predictors for VC in patients with CKD were OPN, intima thickness and CKD stage V. OPN at cutoff point > 144 ng/ml had 93% overall accuracy for the prediction of VC in patients with CKD, and the area under the curve was 0.966.

Conclusion: We concluded that (1) OPN level was significantly higher in hemodialysis patients with diabetic nephropathy stages V than those diabetic patients with chronic kidney disease stage I-IV. (2) OPN level was significantly higher in patients with vascular calcification than in those without. So, OPN can be used as an early marker for the detection of vascular calcification.

Keywords: Diabetic nephropathy, Vascular calcification, Osteopontin, Intima media thickness.

INTRODUCTION

Endothelial dysfunction with subsequent disruption in the normal pathway of vascular hemostasis is a common complication of Type 2 diabetes mellitus (DM) with diabetic nephropathy (DN). Vascular calcification is a characteristic feature of vascular aging that has many cardiovascular complications [(1, 2)].

Osteopontin (OPN) is a multifunctional protein, that has an important role in the pathogenesis of vascular diseases and others (cancer, and renal stones) [(3)]. Currently, OPN is considered the master in vascular remodeling and vascular calcification (VC). Moreover, VC in type 2 diabetic patients is commonly associated with intima-media thickening [(4-6)].

The current research was conducted to assess the applicability of OPN as a marker for the early detection of VC in patients with DN.

PATIENTS AND METHODS

A case-control observational study was conducted at the Internal Medicine and Nephrology in Assiut University Hospitals from 2018 to 2020. A total of 70 patients who had type 2 DM, and were subdivided according to the stage of CKD by MDRD equation [(7)] into two equal groups; group (A): included 35 patients with DN with CKD stage I-IV and group (B): included 35 patients with DN with CKD stage V on hemodialysis. The study included 30 healthy persons without DM who were enrolled as the control group.

Ethical approval:

The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Hospital's Ethics Committee of Assiut University Hospital with ID 1000450. The purpose of the study was explained to all participants, and written informed consent was obtained. The study was registered on *clinicaltrials.gov* with NCT0343145.

Inclusion criteria: Age of patient more than 18 years, patients diagnosed with type 2 diabetes mellitus according to ADA criteria [(8)], and the patient is developing DN as determined by the presence of proteinuria

Exclusion criteria: Type I DM, diabetic patients with significant hepatic, respiratory, and/or cardiac (rheumatic, congestive heart failure) diseases, and chronic kidney disease secondary to causes other than DN (HCV Ab positive, HBsAg positive, autoimmune disease, malignancy, and history of nephrotoxic drugs).

Each patient was subjected to Full history and clinical examination. They were subjected to baseline laboratory data including kidney function tests, liver function tests, complete blood count, and parathormone hormone levels.

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Measurement of OPN:

All blood samples were collected after fasting in a cooling vacutainer and immediately centrifuged. After centrifugation serum was blind coded and stored at -70° until use. Osteopontin levels were measured by the ELISA technique.

Human OPN Quantikine ELISA Kit (R&G, United Kingdom) was used for examination. The assay range of this kit was 0.312- 200.0 ng/mL with less than 0.5% cross-reactivity observed with available related molecules. All determinations were done by duplicating. The mean intra-assay coefficients of variation were <10% of all cases

Echocardiography:

It was done for all patients in both groups to assess valve calcification and diastolic dysfunction. In B-mode, it was performed according to the recommendation of the American Society of Echocardiography on the scanner ACUSON (Siemens, Germany) using a transducer with a frequency of 2.5e5 MHz.

Carotid Duplex Examination:

It was done to assess the intima-media thickness and the presence of atheroma plaque in both groups. All measurements were made by a single examiner who was blinded to the study. High-resolution B-mode ultrasonography with a 10 MHz transducer was used to make the measurements. ***Statistical analysis:***

Data was collected and analyzed by using SPSS (Statistical Package for the Social Science, version 20, IBM, and Armonk, New York). Quantitative data were compared by the Student t-test (between two means) and ANOVA (between more than two means). Nominal data are given as a number (n) and percentage (%). A Chi-square test was implemented on such data. Predictors of VC in patients with CKD were assessed by logistic regression analysis. The receiver operator characteristics curve was used to assess the diagnostic accuracy of osteopontin in the prediction of VC in patients with CKD. The level of confidence was kept at 95% and hence, the P-value was considered significant if < 0.05.

RESULTS

Demographic and baseline laboratory data of studied groups (Table 1):

Body mass index showed a significant difference between the studied groups. Group B had a significantly higher BMI than the group. The frequency of oral hypoglycemic agents was significantly higher among group A (62.9%) in comparison to group B (11.4%). Also, the duration of DM was longer among group B.

Table (1): Demographic data of the studied groups

	Group A (n= 35)	**Group B (n= 35)**	**Control group (n= 30)**	***P***	***P*1**	***P*2**	***P*3**
Age (years)	57.54 ± 7.24	58.51 ± 7.52	57.70 ± 7.53	0.13	0.27	0.12	0.31
Male sex	24 (68.6%)	26 (74.3%)	20 (66.7%)	0.77	0.39	0.54	0.34
Body mass index (kg/m^2)	25.63 ± 3.40	26.94 ± 3.29	25.06 ± 2.98	**0.05**	0.09	0.48	**0.02**
Hypertension	18 (51.4%)	12 (34.3%)	10 (33.3%)	0.23	0.11	0.11	0.57
Therapy of DM Oral hypoglycemic agents Insulin	 22 (62.9%) 13 (37.1%)	 4 (11.4%) 31 (88.6%)			**< 0.001**		
Duration of DM	10.05 ± 4.22	14.48 ± 5.94			**< 0.001**		
Hemoglobin (mg/dl)	10.52 ± 2.82	8.83 ± 1.71	11.48 ± 1.24	**< 0.001**	**< 0.001**	0.06	**< 0.001**
Platelets (10^3/ul)	292.37 ± 14.01	282.25 ± 22.95	327.20 ± 9.88	0.24	0.70	0.21	0.10
Leucocytes (10^3/ul)	6.10 ± 1.14	5.99 ± 1.14	5.89 ± 0.68	0.58	0.30	0.19	0.78
RBS (mg/dl)	278.08 ± 8.35	256.17 ± 8.22	101.03 ± 7.84	**< 0.001**	0.19	**< 0.001**	**< 0.001**
Urea (mg/dl)	56.25 ± 8.67	187.46 ± 7.17	34.07 ± 5.11	**< 0.001**	**< 0.001**	**0.04**	**< 0.001**
Creatinine (mg/dl)	2.18 ± 0.57	6.43 ± 1.33	0.79 ± 0.19	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
HBbA1C (%)	8.53 ± 1.59	7.67 ± 0.95	5.11 ± 0.39	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Albumin/creatinine ratio	1.21 ±0.16	0.51 ± 0.13	0.12 ± 0.06	**< 0.001**	**< 0.001**	**< 0.001**	0.59
eGFR (ml/min/1.73 m^2)	30.57 ± 2.89	8.17 ± 2.1			**< 0.001**		
Albumin (mg/dl)	29.14 ± 5.62	27.08 ± 5.20	33.86 ± 5.87	**< 0.001**	0.16	**< 0.001**	**< 0.001**
Aspartate transaminase (u/l)	20.40 ± 4.34	18.57 ± 4.31	28.73 ± 1.23	**< 0.001**	0.49	**< 0.001**	**< 0.001**
Alanine transaminase (u/l)	23.91 ± 4.50	27.28 ± 5.99	30.80 ± 7.40	0.07	0.24	0.23	0.24
Parathermone (ng/ml)	437.14 ± 16.44	535.28 ± 31.42	51.73 ± 7.81	**< 0.001**	**0.04**	**< 0.001**	**< 0.001**
Osteopontin (ng/ml)	71.91 ± 4.28	168.42 ± 7.56	39.13 ± 1.05	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

Data expressed as frequency (percentage), and mean (SD). *P*-value was significant if < 0.05. **Group A** Diabetic patients with CKD stage I-IV, **and Group B** Diabetic patients with CKD stage V on hemodialysis. **DM**: diabetes mellitus; **eGFR**: estimated glomerular filtration rate; **RBS**: random blood sugar; **HbA1C**: glycosylated hemoglobin. ***P*** compared between different three groups; ***P*1** compared between group A and group B, ***P*2** compared between group A and control group, ***P*3** compared between group B and control group

Echocardiographic and Doppler findings in diabetic patients (Table 2):

Group A had a significantly higher ejection fraction but group B had significantly higher LVEDD. Also, group B had a significantly higher frequency of aortic valve calcification in comparison to group A. Group B had significantly higher intima thickness and a higher frequency of calcified plaque in comparison to group A.

Table (2): Echocardiographic and Doppler findings in diabetic patients

	Group A (n= 35)	Group B (n= 35)	*P*-value
Echocardiographic data			
Aortic valve area (cm)	2.99 ± 0.45	2.96 ± 0.37	0.75
LVEDD (cm)	4.83 ± 0.60	5.27 ± 0.93	**0.02**
LVESD (cm)	3.43 ± 0.93	3.49 ± 0.73	0.75
Ejection fraction (%)	63.03 ± 3.78	58.43 ± 5.40	**< 0.001**
PASP (mmHg)	32.11 ± 6.87	34.57 ± 9.01	0.20
Mitral valve calcification	9 (25.7%)	8 (22.8%)	0.50
Aortic valve calcification	5 (14.3%)	12 (34.3%)	**0.04**
LV diastolic dysfunction	3 (8.6%)	4 (11.4%)	0.50
Doppler findings			
Intima thickness (mm)	0.79 ± 0.19	0.99 ± 0.27	**< 0.001**
CCA plaque	14 (40%)	21 (60%)	0.07
Calcified plaque	11 (31.4%)	20 (57.1%)	**0.02**

Data expressed as frequency (percentage), and mean (SD). *P*-value was significant if < 0.05. **Group A** included diabetic patients with CKD stage I-IV, and **Group B** included diabetic patients with CKD stage V on hemodialysis. **LVEDD:** left ventricular end-diastolic diameter; **LVESD:** left ventricular end-systolic diameter; **PASP:** pulmonary artery systolic pressure; **LV:** left ventricular; **CCA**: common carotid artery

Demographic and laboratory data of the studied patients based on the presence of VC (Table 3):

In the current study, a total of 31 (44.3%) patients had calcified plaque (VC group) while the other patients (55.7%) had no calcified plaque (Non-VC group). Patients with non.VC group had significantly higher hemoglobin and ACR. In contrast, the VC group had significantly higher creatinine and osteopontin.

Table (3): Demographic and laboratory data based on the presence of VC

	Non-VC group (n= 39)	VC group (n= 31)	*P*-value
Age (years)	55.64 ± 7.11	59.90 ± 7.16	**0.01**
Male sex	27 (69.2%)	23 (74.2%)	0.34
Body mass index (kg/m^2)	26.56 ± 2.98	25.93 ± 3.87	0.44
Family history	14 (35.9%)	12 (38.7%)	0.50
Hypertension	13 (33.3%)	17 (54.8%)	0.06
Therapy of diabetes mellitus			**< 0.001**
Oral hypoglycemic agents	25 (64.1%)	7 (22.6%)	
Insulin	14 (35.9%)	24 (77.4%)	
Duration of therapy	1.36 ± 0.48	1.77 ± 0.43	**< 0.001**
Laboratory data			
Hemoglobin (mg/dl)	10.23 ± 2.25	8.97 ± 2.57	**0.03**
Platelets (10^3/ul)	290.05 ± 19.75	283.87 ± 17.21	0.82
Leucocytes (10^3/ul)	5.53 ± 1.74	5.57 ± 1.81	0.92
Random blood sugar (mg/dl)	270.61 ± 9.27	262.74 ± 7.35	0.69
Urea (mg/dl)	107.74 ±8.07	139.61 ± 8.16	0.11
Creatinine (mg/dl)	3.78 ± 0.42	4.97 ± 0.15	**0.03**
HBbA1C (%)	8.31 ± 1.44	7.83 ± 1.27	0.14
ACR	3.74 ± 0.87	1.76 ± 0.1	**0.04**
eGFR (ml/min/1.73 m^2)	23.51 ± 5.56	14.15 ± 3.73	**< 0.001**
Proteins (mg/dl)	65.25 ± 6.51	59.15 ± 8.54	**< 0.001**
Albumin (mg/dl)	28.23 ± 5.66	27.87 ± 6.42	0.85
Aspartate transaminase (u/l)	21.56 ± 4.87	16.87 ± 3.94	**< 0.001**
Alanine transaminse (u/l)	25.97 ± 5.87	25.12 ± 5.95	0.69
Parathermone (ng/ml)	473.97 ± 14.05	501.62 ± 81.57	0.64
Osteopontin (ng/ml)	74.85 ± 8.28	177.16 ± 6.06	**< 0.001**

Data expressed as frequency (percentage), and mean (SD). *P*-value was significant if < 0.05. **VC**: vascular calcification; **eGFR**: estimated glomerular filtration rate; **HbA1C**: glycosylated hemoglobin; **ACR**: albumin/creatinine ratio; **VC**: vascular calcification.

Echocardiographic and doppler findings in diabetic patients based on VC (Table 4):

Patients with VC had significantly higher intima thickness and significantly lower LVEDD and LVESD. Also, those patients had a significantly higher frequency of common carotid artery plaque. Other data showed no significant differences between both groups.

Table (4): Echocardiographic and doppler findings in diabetic patients based on VC

	Non-VC group (n= 39)	VC group (n= 31)	*P*-value
Echocardiography findings			
Aortic valve area (cm)	3.03 ± 0.39	2.91 ± 0.42	0.22
LVEDD (cm)	5.24 ± 0.91	4.81 ± 0.59	**0.02**
LVESD (cm)	3.65 ± 0.97	3.23 ± 0.54	**0.03**
Ejection fraction (%)	60.51 ± 6.42	61 ± 3.03	0.69
PASP (mmHg)	33.89 ± 9.18	32.67 ± 6.41	0.53
Mitral valve calcification	9 (23.1%)	8 (25.8%)	0.50
Aortic valve calcification	10 (25.6%)	7 (22.6%)	0.49
LV diastolic dysfunction	4 (10.3%)	3 (9.7%)	0.62
Doppler findings			
Intima thickness (mm)	0.84 ± 0.22	0.97 ± 0.28	**0.04**
CCA plaque	15 (38.5%)	20 (64.5%)	**0.02**

Data expressed as frequency (percentage), and mean (SD). *P*-value was significant if < 0.05. **VC**: vascular calcification; **LVESD:** left ventricular end-systolic diameter; **PASP:** pulmonary artery systolic pressure; **LV:** left ventricular; **CCA**: common carotid artery

Predictors of vascular calcification in patients with CKD (Table 5):

Based on the current study, the predictors for VC in patients with CKD were osteopontin, intima thickness, and CKD stage V.

Table (5): Regression analysis for prediction of VC in patients with CKD

	Odd's ratio	95% Confidence interval	*P*-value
Age	0.71	0.32-1.65	0.08
Duration of diabetes	1.14	1.11-2.22	0.06
Insulin therapy	0.67	0.33-1.98	0.10
Hemoglobin	1.98	1.43-3.01	0.32
CKD stage V	**2.98**	**2.01-5.55**	**< 0.001**
Total proteins	1.09	1.01-2.87	0.22
Aspartate transaminase	0.98	0.23-1.02	0.43
Intima thickness	**2.11**	**1.78-4.11**	**< 0.001**
LVEDD (cm)	1.76	0.11-3.01	0.91
LVESD (cm)	1.12	0.30-1.01	0.43
Osteopontin	**3.09**	**2.34-6.78**	**< 0.001**

P-value was significant if < 0.05. **VC**: vascular calcification; **CKD**: chronic kidney disease; **LVEDD:** left ventricular diastolic diameter; **LVESD:** left ventricular end-systolic diameter

Diagnostic accuracy of osteopontin in prediction VC in patients with CKD (Table 6-Figure 1):

OPN at cutoff point > 144 ng/ml had 84% sensitivity and 100% specificity with 93% overall accuracy for the prediction of vascular calcification in patients with CKD and the area under the curve was 0.966.

Table (6): Diagnostic accuracy of osteopontin in prediction VC in patients with CKD

Indices	Value
Sensitivity	84%
Specificity	100%
Positive predictive value	100
Negative predictive value	87%
Accuracy	93%
Area under curve	0.966
Cutoff point	> 144
P value	< 0.001

P-value was significant if < 0.05. **VC**: vascular calcification; **CKD**: chronic kidney

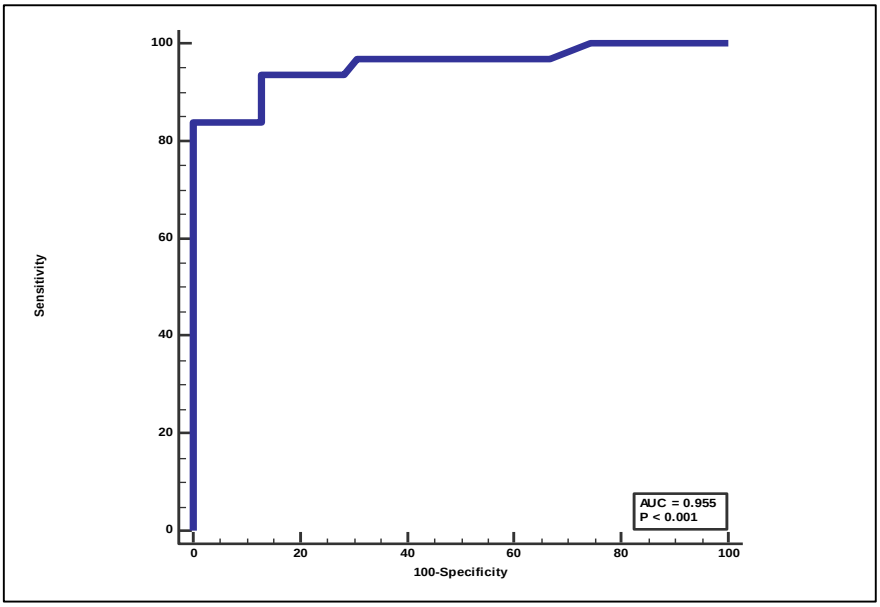

Figure (1): Diagnostic accuracy of osteopontin in prediction VC in patients with CKD

DISCUSSION

VC processes may all be a part of the spectrum of atherosclerosis. VC with its reduced compliance and altered mechanical properties is a predisposing factor for plaque rupture and a predictor of cardiovascular (CV) mortality in patients with DM [(10)]. It's well known that OPN has a role in such issues [(11)].

In the current study, the majority of participants were males and BMI was significantly higher among group B. This was consistent with the study of **Eleftheriadou *et al.*** [(12)] who found that majority of participants were males and BMI was significantly higher in diabetic patients.

In the current study, patients with CKD stage I-IV had a significantly shorter duration of DM and therapy of DM. In line with these results, **Mehrotra *et al.*** [(13)] stated that patients with DN had a longer duration of DM in comparison to those without renal impairment. Also, **Mohan and Sekhar** [(14)] have reported the same findings. But other studies stated that the duration of DM had a nonsignificant association with renal impairment [(15-17)]. This discrepancy between different studies may be explained by different study designs, participants, selection bias, and sample size.

The current study found that patients with CKD stage V had significantly higher levels of OPN in comparison to other groups. These results were similar to the study of **Berezin *et al.*** [(18)] who reported the same findings where OPN was significantly higher in diabetic patients in comparison to the control group. **Abdel-Azeez *et al.*** [(19)] found that OPN was higher level among diabetic patients with coronary artery disease (CAD) in comparison to those without CAD.

Regarding echocardiographic finings, group A had a significantly higher ejection fraction but those with group B had a significantly higher LVEDD. Also, group B had a significantly higher frequency of aortic valve calcification. **Lui *et al.*** [(20)] concluded the same findings where ejection fraction is reduced along with the decline of renal function.

Regarding doppler findings in the present study, patients with stage V CKD (group B) had a significantly higher intima thickness and higher frequency of calcified plaque in comparison to those with stage I-IV CKD (group A). In line with the current results, a study found that OPN levels had a direct correlation with systolic and diastolic blood pressure, body mass index (BMI), and lower high-density lipoprotein [(21)]. **Abdel-Azeez *et al.*** [(19)], stated that OPN is positively correlated with lipid profile and high sensitive CRP.

In the current study, a total of 31 (44.3%) patients had calcified plaque (VC group) while the other patients (55.7%) had no calcified plaque (Non-VC group). Cardiovascular disease is approximately 3 times more frequent in patients with CKD than in other known cardiovascular risk groups and cardiovascular mortality is approximately 10-fold more frequent in patients on dialysis compared to the age- and sex-matched segments of the nonrenal population [(22)].

Also, we found that the vascular calcification group had significantly higher age, longer duration of DM, and longer duration of therapy. Also, vascular calcification was significantly higher among those who received insulin therapy in comparison to those who received OHD (77.4% vs. 64.1%).

The study by **Mehrotra *et al.*** [(13)], reported that VC was more common in males. But all demographic data had no differences between both groups in the previous studies[(19)]. These differences with our study may be secondary to different study designs, sample size, and participants in addition to selection bias.

We found that the VC group had a significantly higher level of OPN. Also, this group had significantly higher intima thickness and significantly lower LVEDD and LVESD with a higher frequency of

CCA plaque. **Abdel-Azeez *et al.*[19]** concluded that patients with CAD had a significantly higher frequency of mitral valve calcification and aortic valve stenosis. Also, another study found that coronary artery calcification score is highly related to IMT of CCA[23]. In line with the current study, **Shoji *et al.* [24]** found significantly increased CCA-IMT compared with healthy controls.

In the current study, the predictors for VC in patients with CKD were OPN, intima thickness, and CKD stage V. Serum OPN at cutoff point > 144 ng/ml had 84% sensitivity and 100% specificity with 93% overall accuracy for the prediction of vascular calcification in patients with CKD and area under the curve was 0.966.

The study of **Abdel-Azeez *et al.* [25]** found that OPN, high sensitive CRP, mitral annular calcification (MAC) grading, and aortic valve sclerosis grading were predictors of coronary atherosclerosis in diabetic patients. **Yan *et al.* [26]** concluded that OPN is associated with the development and degree of nephropathy and coronary affection in diabetic patients and can be used as a predictor of diabetic vasculopathy, while **Berezin and Kremzer[27]** found that OPN can be used as an early marker of coronary artery calcification.

There are some limitations to this study. We believed that a greater cohort in multiple centers would be desirable to improve the power of the study. Also, patients were studied while on medications for hypertension and other metabolic abnormalities associated with renal failure, and we therefore may have underestimated the effects of these factors on VC.

Another limitation is that we measured only CCA-IMT as a structural index of atherosclerosis but measurement of pulse wave velocity as an index of arterial stiffness and arteriosclerosis would provide additional information regarding the effects of these cardiovascular risk factors on functional changes in the arterial wall.

CONCLUSION

OPN level is higher in hemodialysis patients with diabetic nephropathy than those diabetic patients with chronic kidney disease stage I-IV. Also, OPN level is higher in patients with vascular calcification. So, OPN can be used for the early detection of vascular calcification.

Serum osteopontin and assessment of carotid artery intima thickness are non-invasive techniques that would be used for early detection of vascular calcification. Also, multiple center studies with a large number of patients are warranted to confirm the findings of the current study.

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