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ORIGINAL ARTICLE

Serum Osteoinductive Factor as an early Marker of Nephropathy in Type 1 Diabetes Mellitus.

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ABSTRACT

Background: Diabetic nephropathy [DN], as a microvascular complication of both types of diabetes mellitus, is considered a leading cause of end-stage renal diseases in diabetic patients. Microalbuminuria is an early marker of diabetic nephropathy, but some diabetic nephropathy patients with early glomerular influence may present with a normal range of albumin in the urine. Our aim of study is to investigate the relationship between serum Osteoinductive factor and diabetic nephropathy, and its potential use as a diagnostic marker for DN in patients with type 1 DM. this study included a total of 60 type 1 diabetic patients divided to normoalbuminuria, microalbuminuria and macroalbuminuria groups , each group contained 20 patients.

Results: DM duration was significantly higher in macroalbuminuric and microalbuminuric patients than in normoalbuminuric subjects (P=0.00). Systolic and Diastolic Blood Pressure were significantly different among groups showing a higher level in microalbuminuric and macroalbuminuric than normoalbuminuric subjects (p=0.002). Dyslipidemia was significantly more evident in group III (p=0.00) . Macroalbuminuric patients were significantly associated with the highest s.creatinine and the lowest GFR; moreover, the highest level of UACR was significantly evident in the same group (p=0.00).OIF was positively correlated with DM duration, Creatinine and . OIF concentration in microalbuminuric and macroalbuminuric subjects were significantly increased than that in normoalbuminuric group (p=0.00). Significant area under curve(0.893) with significant cutoff [314.5] with sensitivity 80% and specificity 75.0% ,(p=0.00)

Conclusion: OIF was demonstrated in the early stages of DN in T1DM even before appearance of microalbuminuria and increased with progression of diabetic nephropathy. So OIF can be used as a biomarker for the detection of DN in T1DM in microalbuminuric patients.

Keywords: Osteoinductive factor; Diabetes mellitus; Diabetic nephropathy .

INTRODUCTION

Type 1diabetes Mellitus[T1DM] is a chronic metabolic disease of carbohydrate, fat, and protein occurring in childhood as a result of autoimmune destruction of pancreatic beta cells [1]. Diabetic nephropathy[DN], as a microvascular complication of both types of diabetes mellitus, is considered a leading cause of end-stage renal diseases in diabetic patients[2]. Renal cell hypertrophy, glomerular basement membrane thickening, accumulation of extracellular matrix proteins, and Mesangial cell expansion are the features of diabetic nephropathy[3]. Osteoinductive factor[OIF] is a secretory protein that was primarily known to stimulate ectopic bone formation[4]. Osteoinductive factor is incorporated into the normal vascular matrix and has significant roles in lipid metabolism and carbohydrate metabolism[5]. Microalbuminuria is an early marker of diabetic nephropathy, but some diabetic

nephropathy patients with early glomerular influence may present with a normal range of albumin in the urine. so, we need more sensitive markers of early detection of diabetic nephropathy[6]

Yang et al., [2007] reported that microalbuminuria is a standard marker of DN. Unfortunately, this marker easily interfered with excretion, sports, urinary tract infection, hypertension, heart failure, and fever .the epidemiological investigation of diabetic patients showed that 44.3% of patients who got diabetic nephropathy were normoalbuminuric. So, microalbuminuria cannot completely demonstrate the risk of DN[7].

Early detection of diabetic nephropathy is important for management and preventing end-stage renal disease. Diabetic kidney disease is characterized by persistent albuminuria and elevated serum creatinine with a progressive decline in eGFR. Staging of DN depends on various markers, some of them are only effective at the late stages of the disease such as eGFR. Other markers like albuminuria are a dynamic, fluctuating condition[8].

Therefore, OIF has been recently suggested to have a role in the glomerular pathology associated with diabetic nephropathy, moreover; it was studied as a novel biomarker of early diabetic nephropathy[14].So our aim of work is to assess serum Osteoinductive factor as an early marker of nephropathy in T1DM and its correlation with other risk factor .

SUBJECTS AND METHODS

A case-control study was conducted at Internal Medicine Department, Zagazig University Hospitals from September 2018 to August 2019 on type 1 diabetic patients.

Ethical consideration: Written consent was obtained from every patient after explanation of the research. Medical research and ethics committee of Zagazig University approved the study. The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) in 2013 for studies involving humans.

Subjects: This study included 60 type 1 diabetic patients classified to normoalbuminuria, microalbuminuria and macroalbuminuria groups, 20 cases for each. All patients were subjected to medical and clinical history taking and full clinical assessment. Established type 1diabetes mellitus [diagnosed according to WHO criteria [i.e., fasting blood glucose [FBG] ≥ 126 mg/dL, postprandial blood glucose ≥ 200 mg/dL, symptoms of DM with random blood glucose ≥ 200 mg/dL, or A1C ≥ 6.5%], age more than 15 years, under insulin therapy were included in the study. Exclusion criteria were : (1)Acute metabolic disturbance including ketoacidosis, hyperglycemia, hyperosmolar status.(2)Acute severe infection. (3)Patients on hemodialysis.(4) Patients with a bone fracture within the previous 3 months.(5) Autoimmune diseases. (6)Malignancies.(7) Coronary heart disease.(8) Liver diseases. (9)Acute cerebrovascular accidents. (10)Patients with abnormal renal ultrasound beyond diabetic nephropathy(11) Renal bone diseases which start at stage 3 CKD.

Sampling and laboratory investigation

Kidney function tests, fasting blood sugar [FBS], HbA1c, serum total cholesterol, serum triglycerides, serum HDL, serum LDL, UACR. 3 ml of blood collected with tube then centrifuged at 3,000g for 10 minutes, serum samples separated into Opendorph and kept frozen at -80C until tested, Urine samples were withdrawn for albumin, Urine samples were withdrawn for albumin and calculation of urine albumin creatinine ratio [UACR] and estimated glomerular filtration rate [eGFR].

Serum OIF [was determined using an enzyme linked immunosorbent assay [ELISA] kit following the manufacturer's protocols], normal laboratory range of serum Osteoinductive factor (180-290 pg/dl). All routine investigations were done and microalbuminuria was done by Immunoturbidometric assay. The glomerular filtration rate was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation, [10].

STATISTICAL ANALYSIS

Data are expressed as mean value±standard deviation. One-way analysis of variance [ANOVA] was applied to compare means between groups with normally distributed data. Data with a skewed distribution are presented as median±interquartile range. Skewed data with a normal distribution after logarithmic transformation were analyzed using 1-way ANOVA. Skewed data were analyzed using a nonparametric test. Enumeration data were expressed by ratio and percentage with a chi-squared test used for comparison between groups. Correlations between serum level of OIF with UAER and eGFR were analyzed by Pearson correlation analysis. The predictive value of OIF for the risk of early-stage DN was assessed using multivariate logistic regression. Receiver operating characteristic [ROC] analysis was conducted to determine the cut-off value of OIF for predicting early-stage DN. All analyses were performed with Statistical Package for Social Sciences[SPSS version 20.0] . In all statistical tests, differences with $P<.05$ were considered significant.

RESULTS

Demographic informations were collected; the enrolled number of the study was 60 participants, among them 26 males and 34 females.

Table [1] : The mean ages were 20.95±2.09, 21.3±2.19 and 21.4±2.3 for groups I, II, III respectively without a significant difference among groups, also there was no significant difference among studied subjects as regards to BMI, sex or smoking habit according to study design. DM duration was significantly higher in macroalbuminuric and microalbuminuric patients than in normoalbuminuric subjects. There was no significant difference or association regarding HTN but diabetic retinopathy was significantly higher among macroalbuminuric patients.Systolic Blood Pressure and Diastolic Blood Pressure were significantly different among studied groups showing a higher level in microalbuminuric and macro-albuminuric patients than normoalbuminuric subjects.

Table [2]: Dyslipidemia was significantly more evident in group III, without a significant difference between normoalbuminuric and microalbuminuric patients .Concerning Kidney Function Tests, macroalbuminuric patients were significantly associated with the highest s.creatinine and the lowest GFR; more over, the highest level of UACR was significantly evident in the same group. OIF concentration in microalbuminuric patients and macroalbuminuric subjects were significantly increased than that in normoalbuminuric group.

Table [3] : OIF was positively correlated with DM duration, Creatinine and UACR but a significant negatively correlated with eGFR

Figure [1]: Receiver Operating curve (ROC) for OIF marker as a predictor for early nephropathy in diabetic patients. Significant area under curve with significant cutoff [>314.5] with sensitivity 80% and specificity 75.0% for microalbuminuric group.

Table 1: Basic demographic and clinical data distribution among studied groups

			Normoalbuminuria	Microalbuminuria	Macroalbuminuria	F/X^2	P
Age (years)			**20.95±2.09**	**21.3±2.19**	**21.4±2.3**	**1.339**	**0.142**
Body Mass Index(kg/m2)			**25.39±3.39**	**25.95±2.58**	**26.16±6.2**	**0.170**	**0.844**
Diabetes duration (years)			**3.67±1.21**	**7.9±2.46**	**11.85±2.46**	**97.970**	**0.00**
Sex	**Female**	**N**	**13**	**10**	**11**		
		%	**65.0%**	**50.0%**	**55.0%**		
	Male	**N**	**7**	**10**	**9**	**0.95**	**0.62**
		%	**35.0%**	**50.0%**	**45.0%**		
Smoking	**No**	**N**	**19**	**17**	**16**		
		%	**95.0%**	**85.0%**	**80.0%**		
	Smoker	**N**	**1**	**3**	**4**	**2.01**	**0.36**
		%	**5.0%**	**15.0%**	**20.0%**		
Hypertension	**-VE**	**N**	**20**	**19**	**17**		
		%	**100.0%**	**95.0%**	**85.0%**		
	+VE	**N**	**0**	**1**	**3**	**3.75**	**0.15**
		%	**0.0%**	**5.0%**	**15.0%**		
Retinopathy	**-VE**	**N**	**20**	**19**	**15**		
		%	**100.0%**	**95.0%**	**75.0%**		
	+VE	**N**	**0**	**1**	**5**	**7.77**	**0.02**
		%	**0.0%**	**5.0%**	**25.0%**		
Total		**N**	**20**	**20**	**20**		
		%	**100.0%**	**100.0%**	**100.0%**		
SBP (mm Hg)			**116.5±8.12**	**124.5±9.44**	**126.5±8.75**	**7.246**	**0.002**
DBP (mm Hg)			**76.0±8.2**	**83.0±7.32**	**83.0±4.71**	**6.846**	**0.002**

SBP:systolic blood pressure DBP: diastolic blood pressure

Table 2: laboratory data distribution among groups

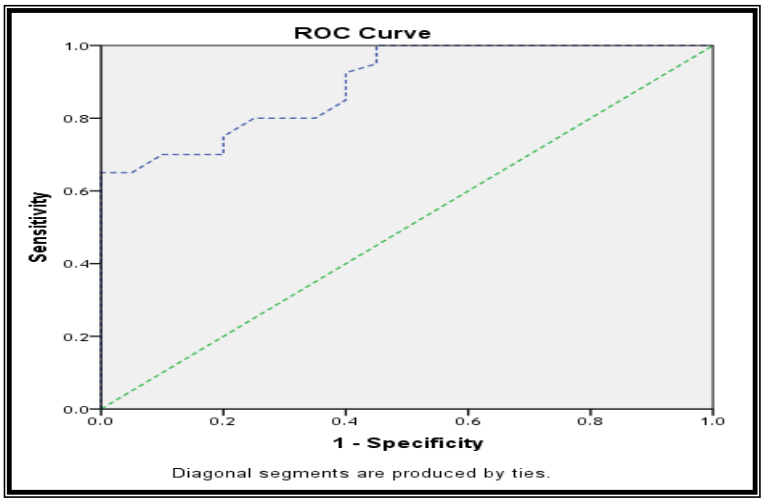
	Normo albuminuria	Micro albuminuria	Macro albuminuria	F	P
Triglycerides(mg/dL)	**100.7±33.74**	**110.3±35.6**	**192.85±68.1**	**17.277**	**0.00**
Cholesterol(mg/dL)	**160.35±30.7**	**170.85±27.1**	**230.85±47.6**	**3.649**	**0.00**
HDL(mg/dL)	**40.05±13.45**	**42.35±10.2**	**37.7±9.94**	**3.135**	**0.051**
LDL(mg/dL)	**68.95±20.24**	**85.3±28.23**	**79.05±16.84**	**2.740**	**0.073**
Creatinine(mg/dL)	**0.76±0.1**	**0.92±0.23**	**2.42±0.12**	**11.422**	**0.00**
Urea(mg/dL)	**31.15±5.65**	**32.4±7.1**	**90.85±5.51**	**3.547**	**0.00**
UACR	**8.32±4.6**	**79.54±39.8**	**625.2±115.5**	**324.465**	**0.00**
e GFR(CKD-EPI)	**116.65±19.68**	**110.0±19.9**	**75.0±10.9**	**10.516**	**0.00**
OIF(pg/dl)	**285.7±25.68**	**328.85±34.2**	**341.55±27.54**	**19.854**	**0.00**

HDL:high density lipoprotein LDL:low density lipoprotein UACR:urinary albumin creatinine ratio eGFR:estimated glomerular filtration rate

Table 3: Correlations between OIF and other parameters:

		OIF
BMI	**r**	**.044**
	p	**.736**
DM duration	**r**	**.508**
	p	**.000**
SBP	**r**	**.452**
	p	**.000**
DBP	**r**	**.425**
	p	**.000**
FBS	**r**	**.132**
	p	**.315**
A1C	**r**	**.185**
	p	**.157**
Triglyceride	**r**	**.109**
	p	**.406**
Cholesterol	**r**	**.178**
	p	**.173**
HDL	**r**	**.059**
	p	**.656**
LDL	**r**	**.075**
	p	**.568**
Creatinine	**r**	**.367**
	p	**.004**
UREA	**r**	**.219**
	p	**.058**
UACR	**r**	**.395**
	p	**.002**
e GFR	**r**	**-.377**
	p	**.003**

Figure 1: Receiver Operating curve (ROC) for OIF marker as a predictor for early nephropathy in diabetic patients



DISCUSSION

Diabetic nephropathy is a chronic complication of long standing and poorly controlled diabetes mellitus, Occurs in 20% to 40% of patients with diabetes [11].The morphologic lesions in type 1 diabetes [T1DM], predominantly affect the glomeruli, with thickening of the basement membrane and mesangial expansion, also the podocytes, renal tubules, interstitium and arterioles are affected, especially at later stages of disease [12].Serum OIF is involved in the development of angiogenesis and atherosclerosis. Vascular endothelial injury and has been shown to be released by vulnerable hemorrhagic carotid and coronary atherosclerotic plaques and may also have prognostic value in patients with coronary artery disease [13].OIF may be a potential biomarker for diagnosing and evaluating the onset and development of DN among DM subjects.[14].

So we conducted this case control study to investigate further the correlation between serum OIF and DN, to investigate the possible mechanism linking OIF and DN, and to assess the value of serum OIF in the early diagnosis and monitoring of DN. We hypothesized that serum OIF is involved in the pathogenesis and development of DN and can be used as a diagnostic marker of DN even before microalbuminuria.

In our study according to study design, there was no significant difference between diabetic patients as regard to age, BMI, sex or smoking habit and This result is similar to result obtained by Wang et al.[14] El-Beblawy et al.[15] and Wei et al [9].

Our results reviewed that DM duration was significantly higher in macroalbuminuric and microalbuminuric patients than in normoalbuminuric subjects and this is in line with the studies of [Rodrigues et al.[16], [El-Beblawy et al[15] who reported that The prevalence of DN associated with T1DM increased with a longer duration of diabetes and usually occurs after 5 years of diabetic duration . Despite that there was no significant difference in history of hypertension in our study patients, measurements of systolic blood pressure and Diastolic blood pressure exhibited significantly higher level in microalbuminuric and macroalbuminuric patients than normoalbuminuric subjects. Variable proposed mechanisms can explain the pathogenic role of diabetes mellitus in development of hypertension involving systemic vascular inflammation , endothelial dysfunction, alterations in atrial natriuretic peptide, and renin-angiotensin system [Stehouwer et al .[17] , Darcan et al.[18],[Wang et al.,[14] who reported that hypertension has an important role in the development of persistent microalbuminuria, supporting the concept that glomerular hypertension is crucial in the initiation and progression of diabetic kidney disease. Hypertension has harmful effects within the glomerulus by inducing impaired autoregulation of the glomerular microcirculation. This consists of vasodilatation of both the afferent and the efferent arteriole, more vigorous effect on the afferent arteriole, resulting in an increase in intraglomerular capillary pressure.[Hostetter, et al[19] and Liu, [20].

Clinically microalbuminuria is the earliest manifestation of diabetic nephropathy [21]Concerning UACR Macroalbuminuric, patients were significantly associated with the highest levels and with the lowest GFR. And this is agree with [Wang et al [14].

Yang et al ,2007 reported that microalbuminuria is a standard marker for DN.but, this marker easily affected by excretion, sports, urinary tract infection, hypertension, heart failure, and fever.

The epidemiological investigation of diabetic patients showed that 44.3% of patients who got diabetic kidney diseases were normoalbuminuric[7]. So, microalbuminuric still cannot completely demonstrate whether patients may get the risk of DN or not.

Early detection of a diabetic kidney disease is crucial to better clinical management and to avoid reaching the end-stage renal disease. Diabetic nephropathy is characterized by persistent albuminuria and elevated serum creatinine with a progressive decline in eGFR. Staging of DN is governed by several markers, some of them are only effective at late stages of the disease such as eGFR. Other markers like albuminuria is a dynamic, fluctuating condition [8] .

The presence of albuminuria is broadly accepted as an indicative of advanced renal structural changes in the kidney reflecting an established state of nephropathy [22]

Our results showed marked deterioration of eGFR in macroalbuminuric patients correlating with diabetic nephropathy progression.

This finding was in agreement in the study published by [Wang et al., 2015] who reported that serum concentrations of OIF were increased in subjects with DN and OIF was a sensitive marker for early microalbuminuria[14]. It showed that serum OIF levels were significantly increased in DN subjects compared with healthy and T2DM subjects. Correlation studies revealed that OIF was positively correlated with creatinine and negatively correlated with eGFR. our study points of strength are that OIF levels were strongly associated with renal function deterioration in subjects with DN. Through carrying out the ROC plots, we found that serum OIF had high sensitive and specificity for the prediction of microalbuminuria.so the results revealed the potential role of serum OIF levels for the onset and development of DN in DM subjects.

In our study, we reported a significant increase in serum OIF level in patients with DN compared to people with T1D.Additionally, clinical markers of Diabetic Nephropathy like UACR and serum creatinine were positively correlated with serum OIF, while glomerular filtration rate reflected by eGFR was negatively correlated with serum OIF.

In our study population, OIF start to rise early in diabetic patients with diabetic duration less than 5 years with normoalbuminuria and progressive rise with progression of nephropathy, suggesting serum OIF as an early marker of nephropathy.

Therefore, measurement of serum osteoinductive factor provides a suitable biomarker for early detection and monitoring of diagnosis of diabetic nephropathy in type 1 DM.

Study Limitations :Small number of patients in the study. Exclusion criteria which used in study protocol rolled out many variables that may affect the results.

Recommendations: Early detection of diabetic nephropathy is recommended to define patients at high risk for developing DN, this may allow prompt interventions and an improved prognosis.

Serum Osteoinductive factor is recommended to be used as an early marker of nephropathy in T1DM.

CONCLUSION

OIF was demonstrated in early stages of DN inT1DM even before appearance of microalbuminuria and increased with progression of diabetic nephropathy.

So OIF can be used as a biomarker for early detection of DN in T1DM in microalbuminuric patients.

Conflict of Interest: Nothing to declare.

Financial Disclosures: Nothing to declare.

REFERENCES

1.Aathira R, Jain V. Advances in management of type 1 diabetes mellitus. World J Diabetes. 2014;5(5):689.

2.Parving H-H, Brenner BM, McMurray JJ V, de Zeeuw D, Haffner SM, Solomon SD, et al. Aliskiren trial in type 2 diabetes using cardio-renal endpoints (ALTITUDE): rationale and study design. Nephrol Dial Transplant. 2009;24(5):1663–71.

3.Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. J Clin Invest. 1984;74(4):1143–55.

4.Hocking AM, Shinomura T, McQuillan DJ. Leucine-rich repeat glycoproteins of the extracellular matrix. Matrix Biol. 1998;17(1):1–19.

5.Shanahan CM, Cary NRB, Osbourn JK, Weissberg PL. Identification of osteoglycin as a component of the vascular matrix: differential expression by vascular smooth muscle cells during neointima formation and in atherosclerotic plaques. Arterioscler Thromb Vasc Biol. 1997;17(11):2437–47.

6.Caramori ML, Fioretto P, Mauer M. Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. Diabetes. 2003;52(4):1036–40.

7.Yang Y, Zhang S, Cui J, Lu B, Dong X, Song X, et al. Diagnostic potential of serum protein pattern in Type 2 diabetic nephropathy. Diabet Med. 2007;24(12):1386–92.

8.Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. Clin J Am Soc Nephrol. 2017;12(12):2032–45.

9.Wei W, Tu M, Huang R, Chen T. Serum osteoinductive factor is associated with microalbuminuria and diabetic nephropathy in type 2 diabetes. Medicine (Baltimore). 2018;97(31).
10.Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. J Am Soc Nephrol. 2009;20(11):2305–13.
11.Persson F, Rossing P. Diagnosis of diabetic kidney disease: state of the art and future perspective. Kidney Int Suppl. 2018;8(1):2–7.
12.Hoshino J, Mise K, Ueno T, Imafuku A, Kawada M, Sumida K, et al. A pathological scoring system to predict renal outcome in diabetic nephropathy. Am J Nephrol. 2015;41(4–5):337–44.
13.Cheng JM, Akkerhuis KM, Meilhac O, Oemrawsingh RM, Garcia-Garcia HM, van Geuns R-J, et al. Circulating osteoglycin and NGAL/MMP9 complex concentrations predict 1-year major adverse cardiovascular events after coronary angiography. Arterioscler Thromb Vasc Biol. 2014;34(5):1078–84.
14.Wang S, Wang Y, Zheng R, Zhao Z, Ma Y. Osteoinductive factor is a novel biomarker for the diagnosis of early diabetic nephropathy. Int J Clin Exp Pathol. 2015;8(3):3110.
15.El-Beblawy NMS, Andrawes NG, Ismail EAR, Enany BE-S, El-Seoud HSA, Erfan MA. Serum and urinary orosomucoid in young patients with type 1 diabetes: a link between inflammation, microvascular complications, and subclinical atherosclerosis. Clin Appl Thromb. 2016;22(8):718–26.
16.Rodrigues TC, Pecis M, Canani LH, Schreiner L, Kramer CK, Biavatti K, et al. Characterization of patients with type 1 diabetes mellitus in southern Brazil: chronic complications and associated factors. Rev Assoc Med Bras. 2010;56(1):67–73.
17.Stehouwer CDA, Henry RMA, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. Diabetologia. 2008;51(4):527.
18.Darcan S, Goksen D, Mir S, Serdaroglu E, Buyukinan M, Coker M, et al. Alterations of blood pressure in type 1 diabetic children and adolescents. Pediatr Nephrol. 2006;21(5):672.
19.Hostetter TH, Rennke HG, Brenner BM. The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. Am J Med. 1982;72(3):375–80.
20.Liu R. The real culprit behind diabetic nephropathy: impaired renal autoregulation? Physiol Rep. 2017;5(5).
21.American Diabetes Association. Standards of medical care in diabetes—2013. Diabetes Care. 2013;36(Supplement 1):S11–66.
22.Drummond K, Mauer M. The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes. Diabetes. 2002;51(5):1580–7.

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