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SERUM OSTEOCALCIN CONCENTRATION AND ITS ASSOCIATION WITH SOME CLINICAL PARAMETERS IN DIABETES MELLITUS

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ABSTRACT

The research included estimate the concentration of osteocalcin and some clinical parameters in control and diabetic patients (Type I and Type II). The results demonstrated that the normal mean of osteocalcin in serum was (28.07 ± 4.79 ng/mL) in control group. also, the results demonstrated a significant decrease in the concentration of osteocalcin in serum of type I and type II diabetic patients compared with control and between type I diabetic patients compared with type II. The results also showed a significant increase in the concentration of all from glucose, MDA, total lipids, total cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic Index, potassium, and in trehalase activity in serum of diabetic patients(type I and II), while found a significant decrease in the concentration of all from insulin, HOMA-β, and thioredoxin, in serum of diabetic patients (type I) compared with control and diabetic patients(type II) and a significant decrease in the concentration of all from GSH, HDL- C, Antiatherogenic Index, sodium, calcium, magnesium and zinc in serum of diabetic patients (type I and II) compared with control. Also, the results showed a significant increase of insulin resistance in type II diabetic patients compared with control and type I diabetic patients, and a significant increase of adiponectin concentration between type I diabetic patient compared with type II and control. Correlation coefficients between osteocalcin and some clinical parameters of control and diabetic patients showed that osteocalcin concentration has a significant negative correlation with concentration of all from glucose, HOMA-IR, total cholesterol, triglycerides, VLDL, LDL-C, atherogenic Index, and in trehalase activity and a significant positive correlation with concentration of all from HOMA-β, thioredoxin, GSH, HDL, antitherogenic Index, sodium, calcium and zinc in control and diabetic patients(type I and II). While osteocalcin has a significant negative correlation with concentration of all from MDA, total lipids and a significant positive

correlation with concentration of all from insulin, adiponectn, and magnesium in control and type II diabetic patients, whereas osteocalcin has a significant negative correlation with potassium concentration in type II diabetic patient.

KEYWORDS: Osteocalcin, thioredoxin, Diabetes, Clinical parameters, Insulin resistance.

INTRODUCTION

Diabetes mellitus is a systemic, chronic metabolic disease characterized by hyperglycemia, dyslipidemia, glucosuria and various concomitant clinical and clinical symptoms, as well as a high risk of early death.[1] Type II diabetes is characterized by a progressive worsening in secretion functions of the produced insulin and the development of peripheral resistance to insulin, rather than a deficit in insulin production.[2]

Studies showed that may be there was relation between diabetes mellitus and discovered hormone (osteocalcin).

Osteocalcin, also called bone Gla-protein or the vitamin k-dependent protein of bone and synthesized predominantly by osteoblasts and in lower way by odontoblasts, is incorporated into the extracellular matrix of bone.[3] In particular, osteocalcin or is a small abundant non-collagenous calcium binding protein, indigenous to the organic matrix of bone dentin and possibly other mineralized tissue, which circulates in the blood It is accepted as a marker of osteoblast activity.[4,5] Research from Columbia University Medical Center demonstrates that bone cells release a hormone called osteocalcin, which controls the regulation of blood sugar (glucose) and fat deposition through synergistic mechanisms, so osteocalcin directs the pancreas' beta cells, which produce the body's supply of insulin, to produce more insulin, at the same time, osteocalcin directs fat cells to release a hormone called adiponectin, which improves insulin sensitivity, this discovery showed for the first time that one hormone has a synergistic function in regulating insulin secretion and insulin sensitivity and that this coordinating signal comes from the skeleton, additionally, osteocalcin enhances the production of insulin-producing beta cells, which is considered one of the best, but currently unattainable, strategies to treat diabetes.[5,6]

Aims of the research

Because there is few previous studies in Iraq about association of osteocalcin with some parameters of diabetes mellitus, so there was suggestion to study its association with some

parameters of diabetes mellitus.

MATERIALS AND METHODS

This study included (70) healthy subjects (35 female, 35 male), with age matching to the patients group as control. Also, (75) patients (37 female, 38 male) with diabetes mellitus from al-waffa center for diabetic patients in Mosul city, were divided into two groups:

Group I: (22) patients from both sexes, their ages range between (less or equal than 15) years old and (16-35) years old with type I diabetes mellitus.

Group II: (53) patients from both sexes, their ages rang (36-49) years old, (50-64)years old and (over or equal than 65) years old with type II diabetes mellitus.

Fasting blood samples were taken and serum were separated and used to estimate the following clinical parameters:

-Osteocalcin: was measured by enzyme linked immunosorbent assay (ELISA) technique[7] using Epitope Diagnostics, Inc kit (USA). This analysis was performed in the immunity laboratory in Al-Salam hospital in Mosul city by using(BIO-TEK INSTRUMENTS, INC), USA.

-Blood glucose: was determined by the enzymatic colorimetric method[8], using Randox kit (United Kindom).

-Insulin: was measured by enzyme linked immunosorbent assay (ELISA) technique[9], using Monobind kit (USA). This analysis was performed in the immunity laboratory in Al- Salam hospital in Mosul city by using(BIO-TEK INSTRUMENTS, INC), USA.

-Homeostasis model assessment of beta cell function (HOMA-β): was calculated from fasting insulin and glucose by the following equation:

HOMA-β(%) = insulin (µU/ mL) ×20/(glucose (mmol/ L)-3.5).[10]

-Homeostasis model assessment of insulin resistance (HOMA-IR): was calculated from fasting insulin and glucose by the following equation:

HOMA-IR=Insulin (µU/ml) x Glucose (mmol/L) /22.5.[11]

-Adiponectin: was measured by enzyme linked immunosorbent assay (ELISA) technique[12], using USBIOLOGICAL kit (USA). This analysis was performed in the immunity laboratory in Al-Salam hospital in Mosul city by using(BIO-TEK INSTRUMENTS, INC), USA.

-Thioredoxin: was found manually by turbidimetric rate determination method manually.[13]

-Trehalase: was found manually by by enzymatic colorimetric method.[14]

-Glutathione: was determined manually according to the modified method of.[15]

-Malondialdehyde(MDA): was determined manually according to the modified method of.[16]

-Total lipids: was found by colorimetric method manually.[17]

-Total cholesterol: was determined by enzymatic colorimetric method[18], using BIOLABO kit (France).

-Triglycerides: was determined by enzymatic colorimetric method[19], using BIOLABO kit (France).

-Very low density lipoprotein-cholesterol (VLDL-C): was calculated by using the following equation: VLDL Conc. (mmol/L) = TG Conc./2.2.[20]

-Low density lipoprotein-cholesterol (LDL-C): was calculated by using the following equation:
LDL Conc. (mmol/L) = Cholestrol Conc.-HDL Conc.-(TGconc./2.2).[21]

-High density lipoprotein-cholesterol (HDL-C): was determined by precipitation method[21], using BIOLABO kit (France).

-Atherogenic Index(AI): was calculated by using the following equation:
Atherogenic Index, AI) = Log(TG/HDL-C).[22]

-Antitherogenic Index(AAI): was calculated by using the following equation: (Antiatherogenic index, AAI) = HDL-C ×100 / (TC -HDL-C).[23]

-Sodium and Potassium: was determined by flame emission spectrophotometry in the education college in Mosul university.[24]

-Calcium, Magnesium, andZinc: was determined by atomic absorption spectrophotometry in the biology department of science college in Mosul university.[24]

-Body mass index (BMI): was calculated as weight in kilogram divided by the squared height in meters.[25]

Data Analysis: The data obtained in the current study was analyzed using Statistical Package for Social Sciences (SPSS)

1. Standard statistical methods were used to determine the mean and standard error.
2. T-test to compare between two parameters.
3. On way Anova (Duncan-test) to compare between more than two parameters.
4. Linear regression analysis [Pearson correlation coefficient (r)] was performed to identify the relationship between different clinical parameters.
5. P-Value≤ 0.05 was considered to be statistically significant.[26]

RESULTS AND DISCUSSION

The research involve estimation of some clinical parameters in diabetic patients compared with control to found the relationship between osteocalcin and some clinical parameters as it is shown in table(1) bellow:

Table (1) demonstrates that diabetic patient (type I and II) have a significantly lower osteocalcin as compared with control. The table also demonstrates that there was a significant lower osteocalcin in type I diabetic patients compared with type II diabetic patients. These results were in agreement with those done by[4,27], the cause of the decreasing of osteocalcin in type I and type II compared with control may be due to osteoblast activity decreasing, or it may be due to insulin secretion decrease which activate the secretion of the hormone active form of osteocalcin.[28] Table (1) demonstrates that type I and II diabetic patients have a significantly higher glucose as compared with control, the elevation of glucose in type I due to the defects in insulin action, but in type II the elevation of glucose related to inability of the cells response to insulin or because defect in function of insulin.[29] While the results in table (1) demonstrates that type I diabetic patients have a significantly lower insulin as compared with type II diabetic patients and control, the cause due to destruction of β-cells in pancreas, so the pancreas does not produce insulin (2). Also the results in table (1) demonstrates type I and II diabetic patients have a significantly lower (HOMA-β) as compared with control, the cause due to its correlation with glucose and insulin (10). Whereas there are no significant difference of HOMA-IR between type I diabetic patients and control, but there are significant higher difference with type II diabetic patients compared with control and type I diabetic patients as it is shown in table (1), the cause due to its

correlation with glucose and insulin (11). Table (1) demonstrates that type I diabetic patients have a significantly higher concentration of adiponectin as compared with type II diabetic patients and control, the cause due to that insulin down-regulates adiponectin synthesis in vitro[30], therefore it is possible that insulin deficiency in patients with type I diabetes may be associated with increased adiponectin synthesis. The table also demonstrates that type II diabetic patients have a significantly lower adiponectin as compared with control. The cause due to fact that adiponectin transcription is regulated by pro-inflammatory cytokines such as TNF-α and IL-6 which are elevated in type II diabetic patients and correlated positively with insulin resistance.[30,31] Also Table (1) demonstrates that type I and II diabetic patients have a significantly lower thioredoxin as compared with control, this reduction might be due to the oxidative stress which lead to free radical production increase and thioredoxin interacting protein (TXNIP) concentration increase which has negative effect on expression and functions of thioredoxin concentration.[32] While Table (1) demonstrates that type I and II diabetic patients have a significantly higher trehalase as compared with control, this might be due to its correlation with glucose.[33] Table (1) demonstrates that type I and II diabetic patients have a significantly lower glutathione as compared with control, this reduction might be due to oxidative stress increasing in diabetes, that lead to increase free radical production and decrease glutathione concentration.[34] Whereas Table (1) demonstrates that type I and II diabetic patients have a significantly higher MDA as compared with control, this might be due to oxidative stress increasing in diabetes, that lead to increase free radical production and lipid peroxidation then increase MDA concentration.[35] Furthormore in table (1) the results demonstrates that diabetic patients (Type I and II) have a significantly higher total lipids compared with control, the cause due to that insulin inhibit lipolysis in adipose tissue and liver because the insulin inhibit the activity of lipase enzyme.[36] Also table (1) demonstrates that type I and II diabetic patients have a significantly higher total cholesterol as compared with control, the cause due to defects in lipid metabolism.[37] Besides there are a significant increase of triglyceride in type I and type II diabetic patients compared with control, the cause due to the enzyme activity of lipoprotein lipase is low in diabetic patients.[37] As well as table (1) demonstrates that type I and II diabetic patients have a significantly higher VLDL-C as compared with control, the cause due to the enzyme activity of lipoprotein lipase is low in diabetes.[38] Also table (1) demonstrates that type I and type II diabetic patients have a significantly higher LDL-C compared with control, the cause may be due to defects in lipid metabolism.[37] While table (1) demonstrates that diabetic patients (type I and II) have a significantly lower HDL-C as compared with control and due to the enzyme activity of

lipoprotein lipase is low in diabetic patients[37,39], which correlated positively with HDL-C.Whereas table (1) demonstrates that type I and type II diabetic patients have a significantly higher atherogenic index (AI) compared with control, the cause may be due to triglycerides concentration increase and HDL-C concentration decrease in type I and type II diabetic patients compared with control.[22] While table (1) demonstrates that type I and type II diabetic patients have a significantly lower antiatherogenic index(AAI) compared with control, the cause may be due to HDL-C concentration decrease and total Cholesterol concentration increase in type I and type II diabetic patients compared with control.[23] Also table (1) demonstrates that type I and type II diabetic patients have a significantly lower sodium as compared with control, this reduction might be due to the glomerular filtration rates, GFR increase in diabetic patients which lead to loss sodium reabsorption in kidney after its clearance (10). Whereas table (1) demonstrates that type I and type II diabetic patients have a significantly higher potassium as compared with control, this increase might be due to effect of some drugs which interact with potassium secretion.[40] While Table (1) demonstrates that type I and type II diabetic patients have a significantly lower calcium as compared with control, this reduction might be due to vitamin D concentration decrease whice facilitate calcium absorption from intestinal.[41] Also table (1) demonstrates that type I and type II diabetic patients have a significantly lower magnesium as compared with control, this reduction might be due to the glomerular filtration rates, GFR increase in diabetic patients which lead to loss magnesium reabsorption in kidney after its clearance (10). Besides table (1) demonstrates that type I and type II diabetic patients have a significantly lower zinc as compared with control, this reduction might be due to the oxidative stress which lead to free radical production increase which lead to zinc concentration decrease in diapetic disease.[42]

Table (1) The concentration of some clinical parameters in control and diabetic patients.

Clinical Parameters	Control mean±S.E	Type I diabetes mean±S.E	Type II diabetes mean±S.E
Osteocalcin (ng/mL)	28.07 ± 4.79	*15.93 ± 3.15	*19.56 ± 3.26
Glucose (mmol/L)	4.96 ± 0.15	*14.52 ± 1.16	*12.38 ± 0.97
Insulin (µU/mL)	10.16 ± 0.09	*3.41 ± 0.10	*8.02 ± 0.32
HOMA-β	140.94 ± 16.04	*7.70 ± 1.15	*19.16 ± 2.79
HOMA-IR	2.25 ± 0.07	2.21 ± 0.17	*4.04 ± 0.30
Adiponectin (µg/mL)	10.99 ± 0.55	*17.68 ± 0.94	*6.53 ± 0.33
Thioredoxin (µmol/L)	3.41 ± 0.53	*1.77 ± 0.26	*1.94 ± 0.36
Trehalase (U/mL)	18.16 ± 0.18	*51.36 ± 0.65	*45.21 ± 0.50

GSH (µmol/L)	12.98 ± 0.28	*6.37 ± 0.30	*4.80 ± 0.31
MDA (µmol/L)	3.39 ± 0.12	*5.24 ± 0.30	*7.17 ± 0.42
Total lipids (TL) (mg/dL)	598.18 ±9.90	*692.25 ± 14.86	*864.96 ± 7.14
Total cholesterol (TC) (mmol/L)	4.64 ± 0.12	*6.14 ± 0.19	*7.44 ± 0.16
Triglycerides (TG) (mmol/L)	1.39 ± 0.008	*2.51 ± 0.11	*3.11 ± 0.04
VLDL-C (mmol/L)	0.63 ± 0.004	*1.13 ± 0.05	*1.41 ± 0.01
LDL-C (mmol/L)	2.71 ± 0.12	*3.98 ± 0.13	*5.13 ± 0.19
HDL-C (mmol/L)	1.33 ± 0.001	*1.05 ± 0.02	*0.93 ± 0.008
Atherogenic Index (AI)	0.02 ± 0.003	*0.37 ± 0.02	*0.49 ± 0.01
Antiatherogenic Index (AAI)	40.01 ± 1.62	*21.87 ± 1.28	*14.76 ± 0.58
Sodium (mmol/L)	139.47 ± 0.60	*106.04 ± 1.06	*99.23 ± 0.68
Potassium (mmol/L)	4.87 ± 0.18	*5.69 ± 0.11	*5.91 ± 0.02
Calcium (mg/100mL)	10.63 ± 0.13	*8.10 ± 0.16	*7.87 ± 0.14
Magnesium (mmol/L)	1.05 ± 0.02	*0.79 ± 0.01	*0.73 ± 0.008
Zinc (µmol/L)	15.97 ± 0.38	*9.86 ± 0.35	*8.91±0.05

*Significant difference at $p \leq 0.05$.

Correlation between the Concentration of Osteocalcin and Some clinical parameters in control and diabetic patients

The results in table (2) demonstrate that there was a significant negative correlation between osteocalcin concentration and glucose concentration in control and diabetic patients (type I and II), the cause may be due to that osteocalcin has role in glucose concentration decrease when glucose concentration rises in blood, through its role in enhance insulin secretion directly vis its binding to GPRC6A receptor on pancreas β cell or indirectly via its role in in enhance glucagon-like peptid-1,GLP-1) secretion from endocrine intestinal cell, aswell as osteocalcin enhanced glucose uptake via its binding to GPRC6A receptor on muscle, liver and adipose tissues.[28] But there was a significant positive correlation between osteocalcin concentration and insulin concentration in control and type II diabetic patients. The cause may be due to role of insulin in enhanced of secretion and releasing the hormone active form (uncarboxylated osteocalcin) to blood via positive feedforward loop which connect bone tissue and pancreas β cell.[43] Also the results in table (2) demonstrate that there was a significant positive correlation between osteocalcin concentration and HOMA-β concentration in control and diabetic patients (type I and II), the cause may be due to that osteocalcin induced β-cell proliferation because of its role in enhancing genes expression that consider a marker of β-cell proliferation(4). While there was a significant negative correlation between osteocalcin concentration and HOMA-IR concentration in control and diabetic patients (type I and II), the cause may be due to that osteocalcin correlate with negative correlation with glucose and BMI.[44] Whereas there was a significant positive correlation between osteocalcin concentration and adiponectin concentration in control and type II

diabetic patients, the cause may be due to that osteocalcin enhance adiponectin secretion from adipose tissue which the latter regulate insulin sensitivity and decrease HOMA-IR, also adiponectin enhanced ostecalcin osteocalcin secretion via endocrine loop which connet between bone tissue and adipose cells.[45] Also the results in table (2) demonstrate that there was a significant positive correlation between osteocalcin concentration and thioredoxin concentration in control and diabetic patients (type I and II), the cause may be due to that thioredoxin interacting protein (TXNIP) which increase in diabetic and it has inhibituion effect on thioredoxin, lead to inhibit osteocalcin carboxylation in bone then osteocalcin concentration decrease in blood.[32] On the other hand, the results also demonstrate that there was a significant negative correlation between osteocalcin concentration and trehalase activity in control and diabetic patients (type I and II), the cause may be due to that osteocalcin correlate with negative correlation with glucose which consider a product from enzymatic reaction of trehalse.[46] While there was a significant positive correlation between osteocalcin concentration and GSH concentration in control and diabetic patients (type I and II), the cause may be due to that the GSH/GSSG redox couple may have a pivotal role in bone remodeling, so GSH/GSSG redox couple increasing lead to increase concentration of mRNA genes which have role in the osteogenic synthesis and differentiation and in osteoclastogenesis.[47] But the results in table (2) demonstrate that there were a significant negative correlation between osteocalcin concentration and MDA concentration in control and diabetic patients (type II), the cause may be due to that osteocalcin has antioxidant and antiinflammatory affects, therefore it inhibit oxidative stress, or may be due to osteocalcin role in enhance adiponectin secretion which has antioxidant affect and decrease from free radical concentration and MDA concentration.[48] The results in table (2) also, demonstrate that there were a significant negative correlation between osteocalcin concentration and total lipids in control and type II diabetic patients, and with concentration of all from total cholesterol, triglycerides, VLDL-C, and LDL-C in control and diabetic patients (type I and II), the cause may be due to that osteocalcin regulates lipids metabolism and decreases adipose mass via its role in enhance adiponectin secretion from adipose tissue which the latter regulate energy metabolism through increases fatty acid oxidation in liver and skeletal muscle, inhibits gluconeogenesis in liver and enhances glucose uptake in skeletal muscle, also osteocalcin increases energy consumption via its role in enhanced genes increasing that consider marker of mitochondria proliferation.[49] While there was a significant positive correlation between osteocalcin concentration and HDL-C concentration in control and diabetic patients (type I and II), the cause may be due to that osteocalcin stimulates insulin

secretion, so the activity of lipoprotein lipase will increase and lead to increase HDL-C ,or the cause may be due to that osteocalcin promotes adiponectin which correlated positively with HDL-C(5). Table (2) demonstrates that there was a significant negative correlation between osteocalcin concentration and atherogenic index(AI) in control and diabetic patients (type I and II), the cause may be due to that osteocalcin correlate by negative correlation with triglycerides and by positive correlation with HDL-C(50). On the other hand, the results demonstrates that there were a significant positive correlation between osteocalcin concentration and antiatherogenic index (AAI) in control and diabetic patients (type I and II), the cause may be due to that osteocalcin correlate by negative correlation with total cholesterol and by positive correlation with HDL- C.[43] Also the results demonstrates that there were a significant positive correlation between osteocalcin and sodium in control and diabetic patients (type I and II), the cause may be due to sodium role in enhance osteoblast activity.[51] While there were a significant negative correlation between osteocalcin and potassium in type II diabetic patients, the cause may be due to that inhibition of voltage-gated potassium channels has role in enhance osteoblast differentiation and mediates osteocalcin regulated insulin secretion in pancreatic β cells.[52] On the other hand, the results demonstrates that there were a significant positive correlation between osteocalcin and calcium in control and diabetic patients (type I and II), the cause may be due to that osteocalcin regulates calcium homeostasis, also calcium may be necessary in osteocalcin structure(5). Also the results demonstrates that there were a significant positive correlation between osteocalcin and magnesium in control and diabetic patients (type II), the cause may be due to magnesium role in osteocalcin synthesis, also magnesium lead to activate GPRC6A receptor of osteocalcin.[53,54] Finally, table (2) demonstrates that there was a significant positive correlation between osteocalcin and zinc in control and diabetic patients (type I and II), the cause may be due to zinc role in bone metabolism, and its role in enhanced insulin-like growth factor, IGF-I) in osteoblast that lead to activate bone remodeling, also zinc lead to activate GPRC6A receptor of osteocalcin.[5,42]

Table (2) The correlation between osteocalcin and clinical parameters in control and type 1 & type 2 diabetes.

Clinical Parameters	Control		Type I diabetes		Type II diabetes	
	r-value	p-value	r-value	p-value	r-value	p-value
Glucose	-0.539*	0.012	-0.600*	0.023	-0.656**	0.006
Insulin	0.476*	0.029	0.468	0.092	0.749**	0.001
HOMA-β	0.567**	0.007	0.665**	0.009	0.770**	0.000

HOMA-IR	-0.468*	0.033	-0.537*	0.048	-0.752**	0.001
Adiponectin	0.667**	0.001	0.422	0.133	0.598*	0.014
Thioredoxin	0.628**	0.003	0.730**	0.003	0.768**	0.002
Trehalase	-0.570**	0.007	-0.667*	0.009	-0.761**	0.001
GSH	0.556**	0.009	0.677**	0.008	0.705**	0.002
MDA	-0.493*	0.023	-0.467	0.092	-0.676**	0.004
Total lipids (TL)	-0.534*	0.013	-0.498	0.070	-0.763**	0.001
Total cholesterol (TC)	-0.465*	0.034	0.774**	0.001	-0.793**	0.000
Triglyceride (TG)	-0.598**	0.004	-0.734**	0.003	-0.624**	0.010
VLDL-C	-0.570**	0.007	0.737**	0.003	-0.625**	0.010
LDL-C	-0.552**	0.009	0.736**	0.003	-0.747**	0.001
HDL-C	0.730**	0.000	0.683**	0.007	0.632**	0.009
Atherogenic Index (AI)	-0.770**	0.000	-0.808**	0.000	-0.701**	0.002
Antiatherogenic Index (AAI)	0.608**	0.003	0.767**	0.001	0.849**	0.000
Sodium	0.489*	0.024	0.661*	0.010	0.623**	0.010
Potassium	-0.358	0.111	-0.482	0.081	-0.622*	0.010
Calcium	0.642**	0.002	0.739**	0.003	0.767**	0.001
Magnesium	0.537*	0.012	0.427	0.128	0.666**	0.005
Zinc	0.550**	0.010	0.757**	0.002	0.734**	0.001

*Correlation is significant at $p \leq 0.05$.

**Correlation is significant at $p \leq 0.01$.

CONCLUSIONS

These results provide evidence of association of osteocalcin with parameters of diabetes mellitus such as glucose, insulin, thioredoxin, trehalase, lipids profile, electrolytes, & trace elements, so it had a role in diabetes mellitus.

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