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Evaluation of Serum Obestatin Level in Rat Model of Diabetic Nephropathy

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Abstract

Background: Diabetic nephropathy is characterized by excessive protienuria and progressive irreversible renal damage. Various mechanisms were suspected to the development of diabetic nephropathy. However, a few studies have reported associations between obestatin and diabetic complications.

Aim of the Work: To evaluate serum OBST in type II diabetic nephropathy rat model and the possible association with the different biochemical and renal parameters.

Material and Methods: Twenty male albino rats were included in the study and divided into two groups: Group (I): Control group, group (II) type II diabetic group rats were fed High Fat Diet (HFD) for 5wks, then the HFD was replaced with normal rodent diet and animals received single intraperitoneal injection of low dose of Streptozotocin (STZ) (35mg/kg). However, rats had been followed up for two weeks (group IIa), four weeks (group IIb) and for eight weeks (group IIc) after induction of diabetes, the following parameters were examined BMI, mean arterial blood pressure, serum OBST, insulin, glucose and calculated insulin resistance, lipids profile, tumor necrosis factor-alpha, angiotensin II levels, urea, creatinine and uric acid. Proteinuria, glomerular filtration rate and renal malondialdhyde level, glutathione peroxidase and superoxide dismutase activities. Histopathological examinations for kidney tissues were also done at the end of experiment.

Results: There were a significant progressive increase in serum levels of OBST, TNF-a and MAP in group II (a, b, c levels) and progressive increase in serum urea, creatinen, UA, ANG II and proteinuria in group IIb and c levels while, group IIa showed insignificant changes. In addition, there were a significant increase in serum glucose, HOMA-IR, total cholesterol, TG, LDL, VLDL and renal MDA, with a significant decrease in serum insulin, HDL, GFR and renal GSH-Px, SOD activities in group IIc level. Moreover, there was a progressive significant decrease in BMI in group II (b and c levels). Furthermore, obestatin levels were positively correlated with all the previously affected parameters in group II at all levels, except with insulin, HDL and GFR which showed a significant negative correlation. While no significant correlation had been found with GSH-Px, SOD activities.

Conclusion: Serum OBST levels were significantly elevated in experimentally induced diabetic nephropathy in rats and positively correlated with most of the measured biochemical and renal parameters except for insulin, HDL-C and GFR was negatively correlated. These findings simplify that OBST can be used as a novel biomarker for diabetes induced complications. As its increase may play a compensatory role in this metabolic disturbance.

Key Words: *T2DM – Rats – Nephropathy – Obestatin.*

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Introduction

DIABETIC Nephropathy (DN) is the major complication of uncontrolled diabetes mellitus which may lead to end stage kidney disease, develops in about 20-40% of type II diabetic patients **[1,2]**. Renal complication in diabetic patients could be attributed to metabolic and hemodynamic changes as a result of disturbance in glucose regulation **[3]**.

Different mechanisms were suspected to the development of diabetic nephropathy and its clinical course including atherosclerotic and inflammatory processes **[4]**. Currently available options for treatment of DN only delay progression of the disease or turn to renal replacement therapy. So, it is important to identify a new biomarkers for early diagnosis and treatment for cases at high risk **[5]**.

Obestatin (OBST) is an interesting but controversial gut hormone **[6]**. It is a 23-amino acid peptide encoded by the same gene that encodes ghrelin **[7]**.

OBST is not only found in the gastrointestinal tract, but also in the spleen, mammary gland tissue, kidney, thyroid gland, circulating plasma and rat White Adipose Tissue (WAT), and adipocytes from both mice and humans, suggesting autocrine/paracrine effects **[8-11]**, it has been reported to bind to and activate the orphan receptor, G Protein-coupled Receptor-39 (GPR39) **[12]**.

OBST plays an important role in various pathological and physiological activities [13]. However, the biological role of OBST remains largely unknown [11], a few studies have reported associations between OBST and diabetic microvascular complications [14,15]. Also, Data regarding serum OBST in T2DM remain conflicting.

OBST has been shown to have multiple functions, including effects on gastrointestinal motility, cell proliferation, glucose and lipid metabolism, endothelial cells as well as anti-inflammatory and cardioprotective actions [10,15].

Because OBST was reported to be associated with diabetes, Insulin Resistant (IR) and cardiovascular risk factors, we supposed that it may also play a role in underlying pathogenic mechanisms which leads to progression of albuminuria in DN.

The aim of the present study is to evaluate serum OBST concentration in type II diabetic nephropathy rat model with protienuria and the possible association of this hormone with the different biochemical and renal parameters.

Material and Methods

Animals: A total number of twenty healthy male albino rats of local strain weighing 190-220g, were obtained from the Animal House of Faculty of Veterinary Medicine Zagazig University. The animals were kept in steel wire cages (5 rats/cage). They were housed in room temperature with normal light/dark cycles. They had free access to food and water throughout the period of the study all rats received care in accordance with the national health guidelines and the study protocol was approved by the Institutional Review Board and Ethics Committee of Faculty of Medicine Zagazig University, the study was conducted in the period from January to April 2018. The rats were accommodated to laboratory conditions for one week before the experiments was started.

Study design: The rats were randomly divided into two main groups:

Group I [control group (n=10)]: Rats were fed on standard chow diet (5% of energy derived from fat, 18% from proteins, and 77% from carbohydrates; 3.3kcal/g). Group II [type 2 diabetic group (n=10)]: In which rats were fed High Fat Diet (HFD) (60.3% of energy derived from fat, 18.4% from protein, and 21.3% from carbohydrates; 5. 1kcal/g) for 5 weeks, then the HFD was replaced with normal rodent diet and animals received single intraperitoneal (ip) injection of low dose of Streptozotocin (STZ) (35mg/kg) (Sigma Aldrich Co.-USA) dissolved in normal saline [16]. After a period of one week of STZ injection, the rats showed marked hyperglycemia with the blood glucose level increased to more than 250mg/dl were included, the rats survived for 8 weeks after induction of diabetes [17], through this period rats were followed-up at three levels according to measured parameters. Group IIa: 2 week after induction of diabetes. Group IIb: 4 weeks after induction of diabetes. Group IIc: 8 weeks after induction of diabetes, seven rats only survived.

Measurement of body weight and length: Each rat was put in closed plastic container and was weighed at the first and the last day of the experiment. Body length was taken as the distance from the nose tip to the anus. Calculation of BMI: BMI = body weight (gm)/length^2 (cm^2), indicator of obesity where the cutoff value of obesity BMI is more than 0.68gm/cm^2 [18]. Measurement of MABP: MABP is measured in millimeters of mercury (mmHg) by Non Invasive Blood Pressure monitor (NIBP 250, Serial No: 21202-108, BIOPAC system, Inc.; USA) [19]. Urine collection: Urine samples were collected for 24 hours by metabolic cages, measured for volume and centrifuged 10 minutes at approximately 3000rpm to remove insoluble materials. The supernatant was kept at 20ºC for further analysis [20]. Urine biochemical analysis: The following urine levels were measured: Total proteins: Was carried out as described by Nishi and Elin [21] using Urinary Protein Assay Kit [Chondrex, Inc. 2607-151 place NE Redmond, WA 98052, USA]. Creatinine: Was carried out as described by Jaffé [22] using Creatinine (Colorimetric) kit [Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia Egypt].

Blood sampling: One cm blood were obtained from retro-orbital venous plexus each time, serum was separated by allowing the blood samples to clot then centrifuged at 3000rpm for 20 minutes, kept frozeen at (–20ºc) until used to measure the serum levels of obestatin, TNF-a, serum urea, creatinine, uric acid, angiotensin II, while glucose, insulin, lipids profile measurement done at the end.

Serum biochemical analysis: The following serum levels were measured:

OBST levels: Was estimated by using rat double-antibody sandwich ELISA kit; (EIAR-OBS; Ray Biotech. Inc., USA) that was purchased from Sigma Aldrich Company. The estimation method was

according to manufacturer's instructions. Insulin levels: According to Temple et al., [23] using Enzyme Amplified Sensitivity Immunoassay (EASIA), using specific insulin kit (BioSource Belgium) and analyzed by spectrophotometers device. Glucose levels: According to Tietz [24] using glucose enzymatic (GOD-PAP)-liquizyme rat Kits (Biotechnology, Egypt). Calculation of (HOMA-IR): According to the equation of Matthews et al., [25] [HOMA-IR = insulin (μU/mL) X glucose (mg/dL)/ 405]. Total Cholesterol (TC) and Triglycerides (TG) according to Tietz [24] using specific cholesterol and triglycerides kits (Spinreact Spain) and analyzed by spectrophotometers device. HDL-c levels according to Tietz [24] by using kits for HDL-cholesterol (BioSource Europe S.A). Low Density Lipoproteins (LDLc) and Very Low Density Lipoproteins (VLDLc) were estimated according to Friedewald et al., [26] formula. LDLc = TC-[HDLc-(TG/5)] & VLDLc = TG/5.

Creatinine levels: As in urine according to Jaffé [22] using Creatinine (Colorimetric) kit [Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia Egypt]. Urea levels: According to Tietz [27] using urea/bun (urease) kit [Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia Egypt]. UA levels: According to Barham and Trinder [28] using uric acid (uricase/ peroxidase) kit: [BioSystems S.A. Quality System certified according to EN ISO 13485 and EN ISO 9001 standards Costa Brava, 30. 08030 Barcelona, Spain]. ANII: Was carried out as described by Kumar et al., [29] using rat angiotensin II Enzyme Immunoassay (EIA) kit (Catalog Number RAB0010, Sigma-Aldrich Co., Egypt). TNF-α level: Was carried out as described by Fernando et al., [30], using commercial ELISA kit, (Catalog Number RAB0480, provided by Sigma-Aldrich Co). Glomerular Filtration Rate (GFR): By using creatinine clearance formula Cockcroft et al., [31].

$$\text{GFR [creatinine clearance] (ml/min)} = \frac{\text{Urine creatinine X Urine volume}}{\text{Serum creatinine X 1440}}$$

Tissue sampling and histopathological examination:

Immediately after collecting blood samples, rats were killed by decapitation after light ether anesthesia. Kidneys were immediately excised, the left one was processed for histopathological studies and the right one was homogenated for biochemical estimations of Malondialdehyde (MDA) level and Superoxide Dismutase (SOD) & Glutathione Peroxidase (GSH-Px) activities. Renal antioxidant system estimation: One small division (200mg) of right kidney was harvested from the rats and accurately weighed. Then, saline was added according to the tissue weight: Saline volume = 1:9 (w/v). Following homogenization at 4ºC by a DY89-electric homogenate (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China), the homogenates were centrifuged at 1,100xg for 15min at room temperature [17].

MDA level: According to the method described by Ohkawa et al., [32]. Superoxide Dismutase (SOD) activity: According to the method described by Kakkar et al., [33]. Glutathione Peroxidase (GSH-Px) activity: According to the method described by Reddy et al., [34].

Statistical analysis: Results were presented as mean ± SD and analyzed using Version 18 SPSS program (SPSS Inc. Chicago, IL, USA). The Analysis of Variance (ANOVA) followed-up with post hoc test were applied to compare the differences among means of groups. Pearsons test was done to detect possibole correlations between serum OBST and all parameters. p-value <0.05 was considered to be significant.

Results

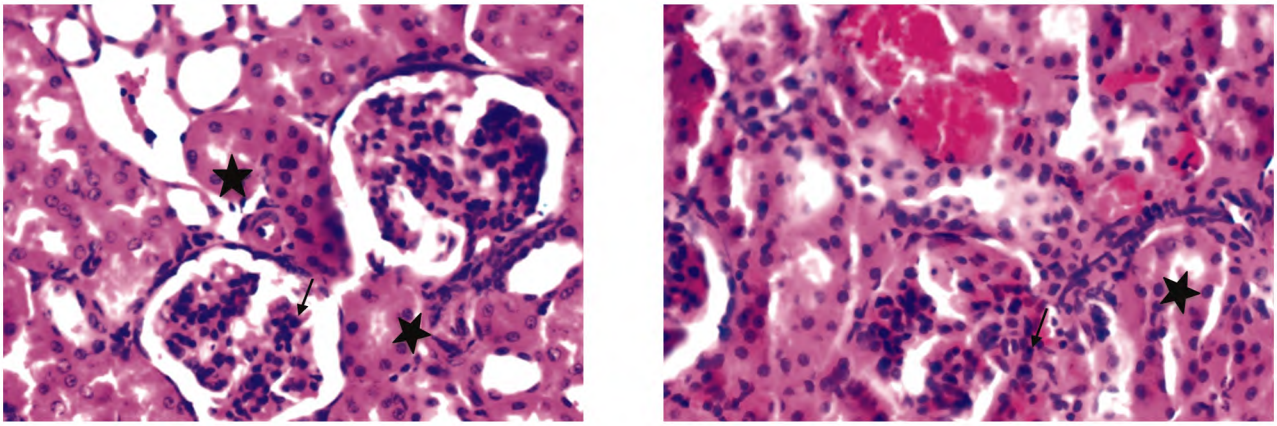
There was a significant progressive increase in serum levels of OBST, TNF-α and MABP in group II (a, b, c stages; p<0.001) compared to each other and group I. And also, there was a significant positive correlation of serum OBST with TNF-α and MABP in these stages rather group I (Table 1).

Although group I rats gradually gained weight, rats in group IIa during first 2 weeks after induction of DM were heavier (p<0.001). However, BMI of group IIb and c at 4 and 8 weeks after induction of diabetes, showed a significant progressive decrease (p<0.001). Additionally serum OBST showed only a significant negative correlation with BMI in group I (Table 1).

Moreover, there was a significant progressive increase in serum levels of urea, creatinen, UA, ANG II and proteinuria in group IIb and c at (4 & 8 weeks, p<0.01, p<0.001 respectively) while group IIa showed insignificant change compared to group I. However, GFR and urine creatinine concentration insignificantly decreased in group IIa (p>0.05) compared to group I. Additionally serum OBST was positively correlated with the previous parameters and negatively correlated with GFR while no correlation with urine creatinine concentration in all stages (2, 4, and 8 weeks) rather than group I (Table 1).

Although, there was a significant increase in serum levels of glucose, TC, TG, LDLc, VLDLc, calculated HOMA-IR and renal MDA levels (p <0.001), there were a significant decrease in serum insulin & HDL-c levels and renal SOD & GSH-Px activities in group IIc (p<0.001) compared to group I. In addition serum OBST was positively correlated with the previous parameters and negatively correlated with insulin and HDL-c levels while no correlation had found with renal SOD & GSH-Px activities in the same group (Table 2).

Regarding histopathology renal tissue in group IIc stage showed sever renal damage with aggregation of inflammatory cells and nephrosclerosis with excessive hemorrhage in the interstitial tissue compared to group I Fig. (1).



(A) (B)

Fig. (1): Photomicrograph of normal renal tissue in control group (A) formed of renal glomeruli (↑ and normal renal tubules (*) (H & E X400). Photomicrograph of renal tissue of a diabetic rat stage c (B) showing atrophic glomeruli with hydrophic degeneration of renal tubular epithelium and areas of hemorrhage (Hematoxyline & Eosin X400).

Table (1): Renal parameters, MABP, serum levels of OBST, ANG II, TNF-α, and BMI of both groups at different levels.

Parameters / Groups	Group I	Group IIa	Group IIb	Group IIc
• Serum OBST (ng/ml)	0.47±.01	0.61±0.03^{a}	0.74±0.04^{a,b}	0.97±.01^{a,b,c}
• BMI (gm/cm^2)	0.56±.06	0.76±0.05^{a}	0.61±0.06^{a,b}	0.47±0.03^{a,b,c}
	r=–0.65*	r=–0.42	r=–0.01	r=–0.46
• Serum urea (mg/dl)	26.09±1.2	25.34±1.9^{NS}	53.5±6.4^{a,b}	84.7±4.7^{a,b,c}
	r=0.28	r=0.69*	r=0.75**	r=0.74*
• Serum creatinine (mg/dl)	0.49±0.05	0.47±0.04^{NS}	1.62±0.11^{a,b}	2.39±.42^{a,b,c}
	r=0.1	r=0.73 *	r=0.67*	r=0.78**
• Serum UA (mg/dl)	0.51±.04	0.5±0.02^{NS}	0.95±0.45^{a,b}	1.88±0.5^{a,b,c}
	r=0.03	r=0.83**	r=0.66*	r=0.69*
• Proteinuria (mg/24h)	48.2±5.2	48.6±6.09^{NS}	190.4±11.4^{a,b}	256.78±8.6^{a,b,c}
	r=0.48	r=0.79* *	r=0.77* *	r=0.72*
• Urine creatinine concentration (mg/ml)	126.8±4.9	124.06±6.2^{NS}	97.1±7.33^{a,b}	81.5±5.8^{a,b,c}
	r=0.03	r=0.12	r=0.02	r=0.53
• GFR (ml/min)	1.53±0.07	1.48±0.11^{NS}	0.74±0.13^{a,b}	0.42±.06^{a,b,c}
	r=0.21	r=–0.63 *	r=–79**	r=–0.81 **
• Serum ANG II (ng/ml)	0.22±.03	0.23±0.01^{NS}	0.29±0.06a.b	0.41±.03^{a,b,c}
	r=0.29	r=0.72*	r=0.067*	r=0.61 *
• TNF-α (pg/ml)	42.6±3.3	51.46±4.13^{a}	58.7±6.04^{a,b}	70.7±6^{a,b,c}
	r=0.16	r=0.79* *	r=0.88**	r=0.75**
• MABP (mmHg)	82.3±6.2	100.2±5.3^{a}	121.36±7.2^{a,b}	130.5±7.3^{a,b,c}
	r=0.02	r=0.69*	r=0.72* *	r=0.76* *

a : Significance vs. group I.
b : Significance vs. group IIa.
c : Significance vs. group IIb.
NS : No Significance.

r : Correlation vs. serum OBST level.
* : Significant (p<0.05).
** : Significant (p<0.01).
*** : Significant (p<0.001).

Table (2): Serum biochemical parameters in group I and group IIc.

Parameters / Groups	Group I	Group IIc
• Serum insulin (μIU/ml)	17.8±1.2	11.6±0.6[a]
	r=0.51	*r*=–0.61 *
• Serum glucose (mg/dl)	85.66±2.5	308.7±24.5[a]
	r=0.41	*r*=0.913***
• HOMA-IR	3.2±.4	13.4±.6[a]
	r=0.2	*r*=0.7*
• TC (mg/dl)	81.1±3.2	180.2±6.5[a]
	r=0.06	*r*=0.72*
• TG (mg/dl)	66.1±4.4	145.7±6.5[a]
	r=0.35	*r*=0.84* *
• HDLc (mg/dl)	41.8±3.1	21.5±3.6[a]
	r=–0.3	*r*=–0.66*
• LDLc (mg/dl)	22.9±4.1	139.4±7.1[a]
	r=0.28	*r*=0.77**
• VLDLc (mg/dl)	9.57±	31.9±3.2[a]
	r=0.03	*r*=0.63 *
• Renal MDA (nmol/gm tissue)	34.3±2.1	69.6±4.7[a]
	r=0.16	*r*=0.77**
• Renal SOD activity (U/mg protein)	10.5±0.6	6.9±0.3[a]
	r=0.03	*r*=0.41
• Renal GSH-Px levels (U/mg protein)	13.5±0.75	10.5±.49[a]
	r=0.5	*r*=0.02

Discussion

DN is characterized by excessive protienuria and leads to progressive and irreversible renal damage, this is directly related to diabetes and/or hypertension [35]. Hence, controlling DN conditions can be usually by overcoming hyperglycemia or proteinuria incidence [5,63].

The induction of type 2DM in this study by the using of HFD feeding produces IR syndrome by increasing plasma free fatty acids as a result of increased influx of triglycerides into the blood [16,37]. After injection of low dose STZ, there were significant hyperglycemia and hypoinsulinemia this could be due to partial destruction of β-cells by STZ induced toxic DNA damage [38]. STZ is transported into the cells by glucose transporter protein, (GLUT2), [39]. STZ is not known by other glucose transporter proteins, and this explains its selective toxic effect on β-cells of the pancreas, as these cells have high levels of GLUT2 [40].

Hyperglycemia, successfully induced renal lesions that were similarly present in human patients with DN and confirmed by histopathological examination where a heavy aggregation of inflammatory cells and nephrosclerosis with excessive glomerular damage and hemorrhage in the interstitial tissue in diabetic rats. These findings are in agreement with the study of Zakkerkish et al., [41], who suggested that hyperglycemia may have a significant role in the progression of DN.

The present rat models of DN suffered from dyslipidemia, hypertension and IR, however, OBST levels were significantly elevated as it is proved to be insulinotropic, antiapoptotic, antinflammtory and promotion of survival in both of β-cells and 3T3-L1 adipocytes and its production has been shown to rise possibly as a compensatory mechanism in IR conditions and obese subjects [10,42]. The signaling pathways involved in these effects include Cyclic Adenosine Monophosphate (cAMP) activation, phosphorylation of survival and proliferative pathways such as phosphatidylinositol 3-kinase, and extracellular signal-related kinase [10,43].

Previous studies have confirmed OBST levels compensatory elevation in patients with obesity, metabolic syndrome, impaired glucose control, T1DM, bulimia nervosa and Chronic Kidney Disease (CKD) [44-46].

In line with these positive findings Zorlu et al., [15] reported that serum OBST levels rise in T2DM patients with albuminuria compared to normal, it might be secondary to the activation of defensive mechanisms against CKD-mediated metabolic and inflammatory disturbance or as possible response against sympathetic nerve stimuli.

Albuminuria actively contributes to endothelial dysfunction which is mostly characterized by low-grade state of systemic inflammation [47].

Additionally, insulin exerts pro-and anti-atherogenic actions on the vasculature. During IR conditions, pathway-specific impairment in phosphatidylinositol 3-kinase-dependent signaling potentially causes inequality between the production of nitric oxide and secretion of endothelin- 1 to promote endothelial dysfunction [48].

Our results are in accordance with the result of Eftekhari et al., [49] which showed an elevated OBST level in children and adult on hemodialysis and explained that elevation to its clearance reduction.

Furthermore, possible inflammatory mechanisms should be considered as an explanation to the rise of serum OBST levels, in COPD patients lei et al., [50] and uremic children Monzani et al., [2]. In addition, the present data demonstrated a significant positive correlation between circulating OBST and serum TNFα compared to control group.

Although OBST dose dependently increased oxidized LDL binding to macrophages, a process that leads to foam cell formation, which is an

essential step in atherogenesis [51]. However, Kellokosk et al., [52] found that in the presence of TNF-a, OBST inhibited Vascular Cell Adhesion Molecule (VCAM-1) expression, suggesting that this protein may adjust the processes participating in atherogenesis, meanwhile concluded as to exhibit antiatherogenic properties in the group of patients with high TNF-a levels and protect them from fatal cardiovascular complication, whereas CKD patients with high TNF-a but low OBST levels are missing such a protection and thus have poorer outcomes [53,54].

Consistently, Zhang et al., [55] demonstrated that OBST was useful as a possible beneficial agent in renal ischemic reperfusion injury. This information proposes that the increase of serum OBST is derived from renal tube in DN to act as one of the renal protective factors.

In contrary to our data, Alhalbouni et al., [56] reported that OBST levels were significantly lower in patients with T2DM, and no correlation was found between its level and inflammatory markers in two chronic inflammatory diseases RA and BD.

The discrepancy between our findings and those of others may be due to species differences, duration, type and/or stages of diabetes or accompanied medications.

Regarding kidney function, there were a significant increase in both of serum urea, creatinine, UA levels and urine flow rate, proteinuria, while the GFR and urine creatinine level were significantly decreased (in duration dependent manner) in diabetic group compared to control group, which indicates development of renal dysfunction. These findings are in accordance with the findings of Zhang et al., [17], who reported that HFD/STZ-induced diabetes affect kidney function.

In addition, we found that serum OBST levels were correlated positively with serum levels of creatinine, urea, UA and proteinuria while this protein correlated negatively to GFR and urine creatinine. This is in line with the results of [2,15,57] who reported higher serum OBST levels were positively correlated with serum urea, creatinin, UA levels and albuminuria in nephropathic patients and inversely correlated with GFR.

Inverse to the current results Lacquaniti et al., [58] found that circulating OBST was significantly lower in uremic children and negatively correlated with serum urea and creatinen while positively associated with GFR.

During this study BMI of rats in control group showed steadily increased. In comparison, those of diabetic group showed significant decrease, which may be due to increased muscle wasting and loss of tissue proteins, and this in line with [59].

While OBST concentrations were negatively correlated with BMI of rats in control group, there was no correlation with BMI of rats inT2DM group at any levels. So, it did not seem to be the reason for elevation of OBST levels in T2DM. This is supported by Gao et al., [60] and Lei et al., [50] who reported that there was a negative association between circulating OBST levels and BMI in normal subjects, but not in patients with chronic atrophic gastritis and COPD. In contrast to the present study, Xin et al., [61] reported OBST was negatively correlated with BMI in CHF and CKD.

In addition, Zhang et al., [14] explored that exogenous OBST decrease food intake in fasting mice. While, Agnew et al., [62] found that OBST exerted no effect on food intake and body weight in rats. From those results we can suggest that OBST expression in some diseases may be different from healthy one.

Regarding, the hyperlipidemia observed in this study could be due to IR that enhance the production of cholesterol, the increase in serum LDLc level may result from non-enzymatic glycosylation of LDLc and may result in decreased LDL clearance [43].

Because the hyperglycemia can undergo auto oxidation and non enzymatic reaction, forming glycated product, which will form oxidants with generation of Reactive Oxygen Species (ROS) [63]. Which it is a risk factor for hyperlipidemia, hypertension and nephropathy [64,65].

ROS can change proteins, lipids and nucleic acids inside the cells, which lead to cellular dysfunction such as metabolic energy loss, cell signaling and transport mechanisms change, genetic mutations, general suppressed biological activity, immune stimulation and inflammation [66,67].

DN group exhibited a significant increase in fasting serum levels of glucose, TC, TG, LDL, VLDL and HOMA-IR they also were positively correlated with serum OBST in diabetic group compared to control group. While, there was a significant decrease in serum insulin and HDL levels in and they were negatively correlated with serum OBST in the same group.

These findings were supported by **[46,68]** who found that the higher OBST levels in long-term type 1 diabetes are a result of a feedback to offset cell death and low insulin gene expression and C peptide secretion. Furthermore, Razzaghy-Azar et al., **[69]** found that the fasting plasma OBST levels in obese children were high, and was significantly correlated with both insulin levels and HOMA-IR, indicating an interrelationship between OBST and insulin.

However, Szentpéteri et al., **[70]** reported that circulating OBST levels were lower in obese patients and negatively correlated with VLDL, TC and TG while positive correlation with HDL and no correlation with other anthropometric parameters.

Also, inverse correlations between circulating OBST and insulin, glucose, leptin, and HOMA-IR have also been reported **[51,71]**. While Lei et al., **[50]** showed no significant correlation was found between plasma OBST and TC, TG, glucose, or insulin.

Being a marker of lipid peroxidation, MDA, is a natural end product produced in intention cell membrane through polyunsaturated lipids degradation **[72]**. In the current study, MDA was significantly increased in the kidney tissue of T2DM rats and negatively correlated with OBST, accompanied by decreased levels of the SOD and GSH-Px activities, in agreement with **[63]**.

Furthermore, in our study ANGII significantly increased in the diabetic group compared to control with significant positive correlation with serum OBST level. This is in agreement with the finding of Al-Qattan et al., **[73]** who reported a significant increase in plasma ANGII in diabetic rats.

However, OBST is reported to inhibit experimental ANGII and dehydration-induced release of vasopressin **[74]**. Hyperglycemia provoked high ANGII concentration and stimulation of over expressed AT 1 receptors lead to sodium retention, vascular resistance, glomerular capillary pressure, and tubulointerstitial cell hypertrophy and hyperplasia associated with extracellular mesangial matrix production **[75]**.

In addition, the present study showed that a significant elevation of MABP in diabetic group compared with control rats and a combined with positive correlation with serum OBST.

Further, hypertension developed in diabetic nephropathy resulted from activation of sympathetic nervous system, renin-angiotensin-aldosterone system which increases reabsorption of sodium and water and results in fluid retention and aggravation of renal filtration and reabsorption **[76]**, endothelial cell dysfunction and increased ROS also reported **[67]**.

Although, Ren et al., **[77]** found a positive association between serum OBST and MABP in both normal pregnancy and those associated with hypertension rather than non pregnant which in line to us, whereas Anderwald-Stadler et al., **[78]** reported plasma OBST levels were negatively correlated with systolic blood pressure in IR patients. Moreover, other studies have reported that OBST induces vascular relaxation, both ex vivo and in vivo in an NO-dependent manner **[79]**.

Summary: Serum OBST levels were significantly elevated in experimentally induced DN in rats and positively correlated with the most measured biochemical and renal parameters except for insulin, HDL-C and GFR was negatively correlated. These findings simplify that OBST can be used as a novel biomarker for diabetes induced complications. As its increase may play a compensatory role in this metabolic disturbance. Further studies are needed to explore the exact mechanism elevates OBST levels in DN.

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تقييم مستوى أوبستاتين فى مصل الدم لنموذج الجرذان المحدث لها إعتلال الكلى السكرى

\الخلفية: يعتبر إعتلال الكلى من المضاعفات الخطيرة لداء السكرى والذى يؤدى إلى تدهور الكلى نهائيا. وتوجد آليات مختلفة لتفسير ذلك. ومع ذلك ذكرت دراسات قليلة عن وجود إرتباط بين مضاعفات الإعتلال السكرى وأوبستاتين.

\الهدف من \البحث: تقييم مستوى أوبستاتين فى مصل دم نموذج الجرذان المستحدث لها إعتلال الكلى السكرى من النوع الثانى وإحتمالية إرتباطه مع مؤشرات الكلى ومختلف المعاملات البيوكيميائية.

\المواد وطرق \البحث: تم إستخدام عشرين ذكرا من الجرذان البيضاء فى الدراسة وقسمت إلى مجموعتين: المجموعة الأولى (I): الضابطة وعددهم (١٠). المجموعة الثانية (II): الجرذان المستحدث لها داء السكرى النوع الثانى وعددهم (١٠) بإستخدام الستربتوزوتوسين (٣٥ (STZ ملجم/كج) وذلك بعد تغذيتها على غذاء عالى الدهون (HFD) لمدة ٥ أسابيع ثم تم إستبدال HFD بالنظام الغذائى للقوارض العادية. بعد ذلك تمت متابعة الجرذان لمدة أسبوعين (المجموعة IIa)، وأربعة أسابيع (المجموعة IIb) وثمانية أسابيع (المجموعة IIc) من حدوث داء السكرى. وفى كل مرة كان يتم فحص وقياس كلا من المعاملات التالية: حساب مؤشر الكتلة ومتوسط ضغط الدم ومستوى أوبستاتين ومستوى أنجيوتنسين-٢ وعامل نخر الورم ألفا واليوريا والكرياتينين وحمض اليوريك ومعدل الترشيح الكبيبى (GFR) بإستخدام تركيز الكرياتينين فى مصل الدم والبول. كما تم قياس معدل تدفق البول وقياس مستوى البروتين والكريتينين به. وبعد قضاء ثمانية أسابيع تم قياس المعاملات السابقة بالإضافة إلى كلا من مستوى الجلوكوز وهرمون الأنسولين ومقاومة الأنسولين فى الدم ومستوى الكوليسترول الكلى والترای جليسيريدات (الدهون الثلاثية) والبروتين الدهنى منخفض الكثافة جدا ومنخفض الكثافة وعالى الكثافة. وأيضا تم عزل الكلى وتحضير أنسجتها للدراسة الميكروسكوبية وقياس مستوى مالون داى الديهيد ومستوى نشاط الجلوتاثيون بيروكسيداز وسوبرأوكسيد ديسميوتاز بها.

\النتائج: أوضحت النتائج أن هناك زيادة تدريجية ذات دلالة إحصائية فى مستويات أوبستاتين ومتوسط ضغط الدم وعامل نخر الورم ألفا بالمجموعة الثانية فى كل المراحل (٢ و٤ و٨ أسابيع من حدوث داء السكرى) أما الزيادة التدريجية فى اليوريا والكرياتينين وحمض اليوريك ومستوى أنجيوتنسين-٢ فى مصل الدم ومستويات البروتين والكرياتينين فى البول حدثت بعد (٤ و٨) أسابيع من المتابعة. بالإضافة إلى ذلك، كان هناك ذات دلالة إحصائية فى مستوى الجلوكوز ومقاومة الأنسولين فى الدم ومستوى الكوليسترول الكلى والترای جليسيريدات والبروتين الدهنى منخفض الكثافة جدا ومنخفض الكثافة فى مصل الدم مع إنخفاض ذى دلالة إحصائية فى مستوى الأنسولين والبروتين الدهنى عالى الكثافة فى مصل الدم ومعدل الترشيح الكبيبى ونشاط الجلوتاثيون بيروكسيداز وسوبرأوكسيد ديسميوتاز الكلوى فى المجموعة الثانية (مستوى C) علاوة على ذلك، كان هناك إنخفاض كبير فى مؤشر كتلة الجسم فى المجموعة الثانية (مستوى B وC). أيضا وجد إرتباط إيجابى بين مستويات أوبستاتين وجميع العوامل المتأثرة سابقا فى المجموعة الثانية على جميع المستويات، بإستثناء الأنسولين ومعدل الترشيح الكبيبى والبروتين عالى الكثافة التى أظهرت علاقة سلبية كبيرة. فى حين لم يتم العثور على إرتباط مع أنشطة الجلوتاثيون بيروكسيداز وسوبرأوكسيد ديسميوتاز.

\الإستنتاج: كانت مستويات أوبستاتين فى مصل الدم مرتفعة بشكل ملحوظ فى إعتلال الكلى السكرى الناجم تجريبيا فى الجرذان وإرتبطت بشكل إيجابى مع أكثر المؤشرات البيوكيميائية ومعاملات الكلى المقاسة بإستثناء الأنسولين وHDL-C وGFR كان الإرتباط سلبيا. هذه النتائج تشير أنه يمكن إستخدام أوبستاتين كمؤشر حيوى جديد للمضاعفات الناجمة عن مرض السكرى. كما أن الزيادة قد تلعب دورا تعويضا فى هذا الإضطراب.