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Osteoprotegerin and Some Trace Elements in Type 2 Diabetic Patients with or without Nephropathy: Effect of Insulin Resistance

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ABSTRACT— We aimed to evaluate the effect of insulin resistance on serum effect of IR on serum OPG and trace elements levels in diabetic nephropathy patients in province of Basrah-Iraq. From 63 volunteers who suffering from T2DM, 31 patients with nephropathy and 32 patients without nephropathy, while 33 normal voleteers were taken as controls. Their fasting insulin hormone and osteoprotegerin were determined by ELISA methods. BMI, glucose urea, creatinine, GFR and Cr.Cl., and homeostasis model assessment for determined of insulin resistance (IR). Mg and Zn were measured by flame atomic absorption spectrometry, while Se in whole blood was determined using hydride generation method. A non-significant change ($p>0.05$) was seen in the level of BMI, significantly decreased ($p<0.01$) in levels of Se, Zn and Mg, while a higher significantly changes ($p<0.01$) were seen in the levels of glucose, insulin, HOMA-IR, urea, creatinine in subjects of type 2 diabetic patients with and without nephropathy, as compared to healthy group. On the other hand, levels of OPG, GFR and Cr.Cl., were highly significantly ($p<0.01$) changes in diabetic patients with nephropathy and significantly ($p<0.05$) changes in patients without nephropathy, compared to the healthy control. Elevated serum OPG and decreased trace elements (Se, Zn and Mg) levels are strongly associated with BMI, insulin resistance and physical activity which can be used as a biomarker of renal dysfunction in diabetic nephropathy thus decreasing the mortality and morbidity.

KEYWORDS: Osteoprotegerin, Trace Elements, Diabetic Nephropathy, Insulin Resistance, Oxidative Stress.

1. INTRODUCTION

Diabetes Mellitus can be illustrated as a group of metabolic morbidness or a metabolic disorder which results from various etiologies in which any diabetic person has hyperglycemia may be due to the pancreas does not make adequate insulin hormone, or because cells do not response to the insulin [1]. The term of insulin resistance (IR) is represented the inability of cells response to the insulin action in transporting glucose from the bloodstream into muscle and tissues. Therefore, it may be developing with obesity and diabetes mellitus, especially with type 2 diabetes [2]. Obesity is usually linked with abnormality in secretion of insulin hormone. Also, it has an ability to increases the resistance to the cellular actions of insulin which may be led to losing the ability of insulin to inhibit glucose output from the liver and to promote glucose uptake in fat and muscle [3; 4]. Diabetes is still an important health problem as a rising number of patients with chronic and poorly maintained diabetes develop diabetic nephropathy "DN" [5]. DN is a kidney disease linked with long-standing hyperglycemia, first discovered in 1936 by P. Kimmelstiel and C. Wilson as inter-capillary glomerulonephritis. The main lineaments of diabetic nephropathy contain the nephrotic syndrome with extravagant filtration of protein into the urine (proteinuria), high blood pressure, and advanced failure of kidney function. In acute cases, DN drives to kidney failure and end-stage renal disease (ESRD) with the

requirement for chronic dialysis or kidney transplantation [6]. Furthermore, DN is still the major cause of all surplus death-rate among type I and II diabetic patients with microalbuminuria, macroalbuminuria or ESRD [7]. Moreover, it starts with glomerular hyper perfusion and kidney hyperfiltration and then goes ahead to microalbuminuria and a minimized glomerular filtration rate (GFR). Present guidelines descibe DN utilizing four main criteria: a fall in kidney function, diabetic retinopathy, proteinuria, and a lowering in GFR [8]. Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor superfamily which acting like a soluble decoy receptor for the receptor activator of nuclear factor-κβ ligand (RANKL) to ban osteoclast activation and bone resorption [9]. The molecule of OPG contains of 401 amino acids; however, the splitting of a 21-amino acid single peptide directs to the forming of a ripe 380 amino acid form, it contains of 4 amino-terminal cysteine rich domains that are structurally like the extracellular fractions of other associates in the TNF receptor superfamily. The carboxyterminal mixes sections 5 and 6 that are death domain symmetrical areas [10]. The OPG molecule contains of three structural scopes operating the biological action. The N-terminal section is a cysteine-rich zone crucial for dimerization and osteoclastgenesis while the C-terminal consists a death domain and a domain for heparin bounding [11]. It is up-regulated in calcified coronary plaques and related with angiographic illness intensity and cardiovascular events separate of classical risk factors [12; 13]. Moreover, OPG has been specified as operating systemic insulin sensitivity and glucose homeostasis [14]. Although the scientific community has started resolving the secrets of the close linkage between IR, OPG and trace elements and their physiological effects, a lot is still remaining to be discovered. In the province of Basrah (southern of Iraq), to date, no study has investigated on IR, OPG and trace elements and their effects on DN patients. Therefore, present study is focused on the objective of assessing the effect of IR on serum OPG and trace elements levels in diabetic nephropathy patients in province of Basrah-Iraq.

2. Patients and Methods

2.1. Subjects

The present study is a cross-sectional clinical experiment. Samples were gathered from the diabetes and endocrine glands center in Al-Mawany Teaching Hospital in Basrah Province-Iraq during the period from August 2019 until February 2020. As much as 188 subjects (men and women) were participated in the present study aged between 36-65 years old. Ninety-two of subjects (52 patients and 40 healthy controls) were dropped out from the study due to enable to follow up study. Final 63 subjects (men and women) who suffering from T2DM were selected to share in this study and they divided into two groups: the first group was consisted of 31 patients who suffering from diabetic nephropathy (15men and 16women) while the second group was consisted of 32 patients who not suffering from diabetic nephropathy (15men and 17women). These subjects were matched with 33 healthy controls (16 men and 17 women). Patients on insulin, hypertension, smokers, alcoholics, tobacco chewers, abnormal urinary sediment, urinary tract infection, renal transplantation, history of other kidney disease and active or chronic persistent infection or inflammatory disorders, neoplastic disorders, uncontrolled thyroid disorders, severe liver dysfunction, HIV infections, history of acute myocardial infarction, stroke, and occlusive peripheral vascular disease, congestive heart failure, pancreatic diseases, hormonal abnormalities and genetic syndromes were excluded from this study. An informed written approval was gotten from all subjects. A full history was taken from all subjects in addition to clinical examination with special concern on age, gender, body mass index, duration of DM & DN and co-morbidities. The patients are already diagnosed as T2DM according to the WHO [15].

2.2. Samples

All blood samples were gathered in the morning between 09:00 and 10:00 a.m after a 12 hours fasting time and a 30 minutes of rest in the supine position. Blood was collected at 2-3 days of menstrual cycle for women. Fresh venous blood (10 mL) were obtained from all volunteers by vein punch then splited into two parts, the first part was 1 mL and it was added into EDTA containing polypropylene tubes and shook gently to be utilized for the determination of the concentration of Selenium (Se). The rest blood was moved to plain tube (without anticoagulant) which admitted clotting for 20 minutes at room temperature. After the blood had clotted, it was moved into a centrifuge and spun at 3000 RPM for 20 minutes to get the serum. The collected serum immediately utilized in the determination of glucose, insulin, urea, creatinine, Zn, Mg and osteoprotegerin. The rest serum was stored in deep freezing at (-20ºC) until used.

2.3. BMI calculation

BMI was calculated by the standard BMI equation [16]:
BMI (kg/m^2) = weight (kg)/height (m^2)

2.4. Routine Methods of Biochemical Estimation

Routine parameters (glucose, urea and creatinine) were assayed on UV-Vis Spectrophotometer (UV-EMC-LAB, Duisburg, Germany) by using the following kits: Glucose kit (Randox, County Antrim, UK, Cat.No.: GL364), urea kit (Linear, Barcelona, Spain, Cat.No.: 1156015) and creatinine kit (Randox, County Antrim, UK, Cat.No.: CR 511/S).

2.5. Assay of serum insulin

Serum insulin was assayed by human insulin enzyme-linked immunosorbent assay "ELISA" kit (Calbiotech, California, USA, Cat.No: IS130D). Sandwich ELISA technique was applied. The level of human insulin was assayed using a standard curve.

2.6. Assay of serum OPG

Serum OPG was assayed by human insulin enzyme-linked immunosorbent assay "ELISA" kit (BT-Lab, Shanghai, China, Cat.No.: E1558Hu). Sandwich ELISA technique was applied. The level of human OPG was assayed using a standard curve.

2.7. Assay of whole blood Selenium

Selenium was determined by Flame Atomic Absorption Spectrometer with Homemade Hydride Generation System (Shimadzu, Tokyo, Japan) [17].

2.8. Assay of serum Zinc and Magnesium

Serum Zinc (Zn) and Magnesium (Mg) were determined by using GBC 933 Plus flame atomic absorption spectrometry "AAS" (GBC, Braeside, Australia) [18].

2.9. Insulin resistance calculation

Insulin resistance (IR) was calculated by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) equation [16]:
HOMA-IR = Fasting insulin (μIU/mL) x Fasting glucose (mg/dL) / 405

2.10. GFR calculation

GFR was calculated by the Modification of Diet in Renal Disease Study (MDRD) equation [19]:

GFR (mL/min/1.73 m2) = 186 × Serum Cr-1.154 × age-0.203 × 1.212 (if patient is black) × 0.742 (if female)

2.11. CrCl calculation

CrCl was calculated by the Cockcroft-Gault Equation [19]:
CrCl (mL/min) = (140 – age) × (weight, kg) × (0.85 if female) / (72 × Serum Cr)

2.12. Ethical issues

The study obtained an ethical approval from college of science in Basrah University (7/54/1290), and an informed consent was gotten from each participant after clarification of the procedures in full details. The informed agreement and ethical guidelines were obeyed according to the Declaration of Helsinki for year 2000.

2.13. Statistical Analysis

For statistical analysis, results are analyzed by employing the SPSS software (Version 22) and the values were described as "mean ± standard deviation (SD). Pearson's correlation analysis was executed. All comparisons were 2-tailed and counted highly statistically significant when ($p<0.01$), statistically significant when ($p<0.05$) and statistically non-significant when ($p>0.05$).

3. Results

Ninety-six subjects were shared in this study. From total 63 type 2 diabetics patient's subjects, 31 subjects were suffered from nephropathy (15men & 16women), meanwhile the other 32 were free from nephropathy (15men & 17women). As much as 33 healthy control subjects were considered as control group (16men & 17women). The general characteristics of all subjects participated in the present study were given in Table1.

Table 1. The demographic characteristics of the present study

The Characteristics		Type 2 Diabetic Patients		Healthy Control
		without NP	with NP	
Total Subjects No.		31	32	33
Age (Years) (Mean ± SD)		57.26±4.47	53.94±4.74	51.70±5.07
DM Duration (Years) (Mean ± SD)		23.27±4.38	17.47±4.52	-
NP Duration (Years) (Mean ± SD)		7.32±1.74	-	-
Demographic Area	Urban	28	27	29
	Rural	3	5	4
Educational Background	Learned	25	24	26
	Illiterate	6	8	7
Smoking Habits	Positive	0	0	0
	Negative	31	32	33
Food Habits	Vegetarian	5	6	8
	Non-Vegetarian	26	26	25
Employment Status	Employed	19	14	28
	Not Employed	12	18	5
Specimens Gender	Men	15	15	16
	Women	16	17	17

Results mention in (Table 2) showed that a non-significant change ($p>0.05$) was seen in the level of BMI, significantly decreased ($p<0.01$) in levels of Se, Zn and Mg, while a higher significantly changes ($p<0.01$) were seen in the levels of glucose, insulin, HOMA-IR, urea, creatinine in subjects of type2 diabetic patients with or without nephropathy, as compared to healthy group. On the other hand, levels of OPG, GFR and Cr.Cl., were highly significantly ($p<0.01$) changes in diabetic patients with nephropathy and significantly

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(p<0.05) changes in patients without nephropathy, compared to the healthy control.

Table 2. The levels of total parameters measured in the present study. The values are the Mean ± SD

Biomarkers	Type 2 Diabetic Patients n=63 (Men & Women)										Healthy Control n=33 (Men & Women)
	with NP n=31 (Men & Women)					without NP n=32 (Men & Women)					
	Mean ± SD	SE	Range	95% CI		Mean ± SD	SE	Range	95% CI		Mean ± SD
				Lower	Upper				Lower	Upper	
BMI (Kg/m²)	30.63±1.35	0.24	28.7-34.1	26.88	34.38	30.60±1.79	0.32	26.3-34.6	25.63	35.57	30.79±1.46
Glucose (mg/dL)	167.67±5.92**	1.06	159-180	151.23	184.1	163.38±5.57**	0.98	151-174	147.91	178.84	101.9±4.8
Insulin (µU/mL)	28.99±2.06**	0.37	24.65-33.47	23.27	34.7	28.08±2.12**	0.37	24.32-32.02	22.19	33.96	11.09±1.57
HOMA-IR	12.01±1.08**	0.19	9.9-14.9	9.01	15.01	11.33±1.04**	0.18	9.2-13.8	8.45	14.22	2.80±0.53
Urea (mg/dL)	56.72±4.06**	0.73	51.01-65.75	45.45	67.99	33.20±4.03*	0.71	24.9-40.9	22.02	44.39	27.27±3.98
Creatinine (mg/dL)	1.60±0.22**	0.04	1.35-1.96	0.99	2.21	1.05±0.11*	0.02	0.89-1.29	0.75	1.36	0.87±0.07
GFR (mL/min/1.73m²)	41.70±5.25**	0.94	36.8-58	27.12	56.27	67.35±6.97*	1.23	56.6-83.8	48	86.7	85.48±8.96
CrCl (mL/min)	64.47±7.45**	1.34	54-85	43.79	85.15	98.03±10.45*	1.85	81-116	69.02	127.04	128.12±10.46
OPG (ng/mL)	3.25±0.38**	0.07	2.33-4.01	2.20	4.30	2.65±0.21*	0.04	1.93-3.37	2.07	3.24	1.87±0.27
Se (ng/mL)	57.29±6.64**	1.19	49.27-67.65	38.86	75.72	64.87±5.91**	1.04	58.47-73.64	48.46	81.27	86.29±6.80
Zn (µg/mL)	0.76±0.24**	0.04	0.42-1.31	0.09	1.43	0.86±0.19**	0.03	0.53-1.25	0.33	1.39	1.29±0.43
Mg (µg/mL)	14.97±2.77**	0.50	8.71-18.95	7.28	22.66	17.28±2.81**	0.50	12.43-21.75	9.48	25.08	21.74±3.65

Data are presented as mean ± SD, SE: Standard Errors; n: Number of the subjects; Range: is the difference between the highest and lowest values in the set; 95% C.I: Confidence limits (Lower and Upper); p-value: N.S ($p > 0.05$), S ($p < 0.05$), HS ($p < 0.01$) indicate the level of significance in comparison with the corresponding control value.

Data obtained in (Table3) revealed a non-significant change (p>0.05) in BMI level (Men: 31.27 ± 1.67, 30.30 ± 2.56 Vs. 31.56 ± 0.25 kg/m²; Women: 30.12 ± 0.51, 30.86 ± 0.38 Vs. 30.08 ± 1.74 kg/m²) respectively, in type 2 diabetic patients with or without nephropathy patients compared to control. On the other hand, levels of glucose, insulin and HOMA-IR were significantly (p<0.01) increased (Men: 172.73 ± 3.35, 168.60 ± 3.09 Vs. 104.26 ± 3.82 mg/dL; Women: 162.38 ± 2.34, 158.76 ± 2.15 Vs. 99.68 ± 4.56 mg/dL), (Men: 29.06 ± 2.72, 28.17 ± 2.37 Vs. 11.50 ± 1.81 µU/mL; Women: 28.78 ± 1.14, 27.99 ± 1.88 Vs. 10.70 ± 1.17 µU/mL) and (Men: 12.41 ± 1.31, 11.74 ± 1.11 Vs. 2.98 ± 0.58; Women: 11.54 ± 0.55, 10.98 ± 0.82 Vs. 2.64 ± 0.41), respectively in type 2 diabetic patients with or without nephropathy patients compared to healthy control. Same Table 3 reflect that levels of urea, creatinine and OPG were significantly (p<0.01) higher (Men: 59.31 ± 3.97 Vs. 29.63 ± 4.05 mg/dL; Women: 53.94 ± 1.80 Vs. 25.04 ± 2.28 mg/dL), (Men: 1.77 ± 0.19 Vs. 0.93 ± 0.05 mg/dL; Women: 1.43 ± 0.05 Vs. 0.81 ± 0.05 mg/dL) and (Men: 3.25 ± 0.12 Vs. 1.96 ± 0.35 ng/mL; Women: 3.19 ± 0.51 Vs. 1.79 ± 0.09 ng/mL), respectively in diabetic patients with nephropathy, as compared to the healthy control. From the other hand, there were a significant increased (p<0.05) in levels of urea, creatinine and OPG in subjects of type2 diabetic patients without nephropathy, when compared with the healthy group (Men: 36.70 ± 2.59 Vs. 29.63 ± 4.05 mg/dL; Women:

30.12 ± 2.11 Vs. 25.04 ± 2.28 mg/dL), (Men: 1.15 ± 0.08 Vs. 0.93 ± 0.05 mg/dL; Women: 0.97 ± 0.05 Vs. 0.81 ± 0.05 mg/dL) and (Men: 2.71 ± 0.15 Vs. 1.96 ± 0.35 ng/mL; Women: 2.61 ± 0.24 Vs. 1.79 ± 0.09 ng/mL), respectively. Furthermore, a significant ($p<0.01$) decreased were seen in levels of Se, Zn and Mg (Men: 63.59 ± 3.41, 70.92 ± 1.85 Vs. 92.81 ± 1.08 ng/mL; Women: 50.88 ± 1.95, 59.53 ± 1.72 Vs. 80.14 ± 3.29 ng/mL), (Men: 0.80 ± 0.26, 0.96 ± 0.16 Vs. 1.59±0.30 µg/mL; Women: 0.70 ± 0.22, 0.77 ± 0.18 Vs. 1.01 ± 0.32 µg/mL) and (Men: 16.71 ± 1.38, 19.93 ± 1.12 Vs. 23.42 ± 3.78 µg/mL; Women: 12.95 ± 2.51, 14.94 ± 1.43 Vs. 20.15 ± 2.68 µg/mL), respectively in type2 diabetic patients with or without nephropathy, as compared to healthy control. Moreover, results in (Table 3) show a highly significantly decreases ($p<0.01$) in levels of GFR and CrCl in subjects of type 2 diabetic patients with nephropathy, compared to control (Men: 43.24 ± 6.92 Vs. 91.19 ± 6.86 mL/min/1.73m²; Women: 40.40 ± 2.17 Vs. 80.11 ± 7.22 mL/min/1.73m²) and (Men: 66.53 ± 8.83 Vs. 130.31 ± 12.13 mL/min; Women: 62.88 ± 5.31 Vs. 126.06 ± 8.06 mL/min), respectively. This study showed, a significant decreases ($p<0.05$) was seen in GFR level in type 2 diabetic subjects without nephropathy (70.83±7.32 mL/min/1.73m²) in men and (64.28±4.91 mL/min/1.73m²) in women compared with that of control were (91.19±6.86 mL/min/1.73m²) in men and (80.11±7.22 mL/min/1.73m²) in women and a significant decreases ($p<0.05$) was seen in level of CrCl in type 2 diabetic subjects without nephropathy (96.07±10.91 mL/min) in men and (99.76±9.71 mL/min) in women compared with that of control were (130.31±12.13 mL/min) in men and (126.06±8.06 mL/min) in women, as shown in (Table 3), respectively.

Table 3. The levels of BMI, OPG HOMA-IR and some trace elements in men and women of healthy control and type 2 diabetic patients. The values are the Mean ± SD

Biomarkers	Gender	Type 2 Diabetic Patients										Healthy Control
		with NP					without NP					
		Mean ± SD	SE	Range	95% CI		Mean ± SD	SE	Range	95% CI		Mean ± SD
					Lower	Upper				Lower	Upper	
BMI (Kg/m²)	Men	31.27±1.67	0.43	28.7-34.1	26.64	35.91	30.30±2.56	0.66	26.3-34.6	23.19	37.41	31.56±0.25
	Women	30.12±0.51	0.13	29.8-32.1	28.70	31.53	30.86±0.38	0.09	29.6-31	29.81	31.92	30.08±1.74
Glucose (mg/dL)	Men	172.73±3.35**	0.86	162-180	163.43	182.03	168.60±3.09**	0.80	158-174	160.02	177.18	104.26±3.82
	Women	162.38±2.34**	0.59	159-171	155.88	168.87	158.76±2.15**	0.52	151-163	152.80	164.73	99.68±4.56
Insulin (µU/mL)	Men	29.06±2.72**	0.70	24.65 - 33.47	21.51	36.61	28.17±2.37**	0.61	24.32 - 32.02	21.59	34.75	11.50±1.81
	Women	28.78±1.14**	0.29	26.84 - 30.62	25.62	31.95	27.99±1.88**	0.46	24.69 - 31.13	22.77	33.21	10.70±1.17
HOMA-IR	Men	12.41±1.31**	0.34	9.9-14.9	8.78	16.05	11.74±1.11**	0.29	9.5-13.8	8.66	14.82	2.98±0.58
	Women	11.54±0.55**	0.14	10.5-12.9	10.01	13.06	10.98±0.82**	0.20	9.2-12.5	8.70	13.25	2.64±0.41
Urea (mg/dL)	Men	59.31±3.97**	1.03	52.87 - 65.75	48.29	70.33	36.70±2.59*	0.67	32.5-40.9	29.51	43.89	29.63±4.05
	Women	53.94±1.80**	0.45	51.01 - 56.86	48.94	58.93	30.12±2.11*	0.51	24.9-37.09	24.26	35.97	25.04±2.28
Creatinine (mg/dL)	Men	1.77±0.19**	0.05	1.38-1.96	1.24	2.30	1.15±0.08*	0.02	1.01-1.29	0.93	1.37	0.93±0.05
	Women	1.43±0.05**	0.01	1.35-1.50	1.29	1.56	0.97±0.05*	0.01	0.89-1.05	0.83	1.11	0.81±0.05

GFR(mL/min/1.73 m²)	Men	43.24±6.92**	1.79	36.8-58	24.03	62.45	70.83±7.32*	1.89	60-83.8	50.51	91.15	91.19±6.86
	Women	40.40±2.17**	0.54	37-44.1	34.38	46.42	64.28±4.91*	1.19	56.6-72.9	50.65	77.91	80.11±7.22
Cr. Cl. (mL/min)	Men	66.53±8.83**	2.28	58-85	42.02	91.05	96.07±10.91*	2.82	81-113	65.78	126.35	130.31±12.13
	Women	62.88±5.31**	1.33	54-71	48.13	77.62	99.76±9.71*	2.36	85-116	72.81	126.72	126.06±8.06
OPG (ng/mL)	Men	3.25±0.12**	0.03	3.04-3.46	2.92	3.58	2.71±0.15*	0.04	2.47-2.96	2.29	3.13	1.96±0.35
	Women	3.19±0.51**	0.13	2.33-4.01	1.78	4.61	2.61±0.24*	0.06	1.93-3.37	1.94	3.27	1.79±0.09
Se (ng/mL)	Men	63.59±3.41**	0.88	62.12 - 67.65	54.13	73.06	70.92±1.85**	0.48	69.34 - 73.64	65.78	76.05	92.81±1.08
	Women	50.88±1.95**	0.49	49.27 - 55.08	45.46	56.29	59.53±1.72**	0.42	58.47 - 64.21	54.76	64.31	80.14±3.29
Zn (µg/mL)	Men	0.80±0.26**	0.07	0.50-1.31	0.08	1.52	0.96±0.16**	0.04	0.55-1.25	0.52	1.40	1.59±0.30
	Women	0.70±0.22**	0.06	0.42-1.12	0.09	1.32	0.77±0.18**	0.04	0.53-1.13	0.27	1.27	1.01±0.32
Mg (µg/mL)	Men	16.71±1.38**	0.36	14.47 - 18.95	12.88	20.54	19.93±1.12**	0.29	18.11 - 21.75	16.82	23.04	23.42±3.78
	Women	12.95±2.51**	0.63	8.71-16.97	5.98	19.92	14.94±1.43**	0.35	12.43 - 17.33	10.97	18.91	20.15±2.68

Data are presented as mean ± SD, SE: Standard Errors; n: Number of the subjects; Range: is the difference between the highest and lowest values in the set; 95% C.I: Confidence limits (Lower and Upper); p-value: N.S ($p > 0.05$), S ($p < 0.05$), HS ($p< 0.01$) indicate the level of significance in comparison with the corresponding control value.

4. Discussion

To the best of our knowledge, this is the first study climbed on the objective of assessing the effect of IR on serum OPG and some trace elements levels in diabetic patients with and without nephropathy in Basrah province (southern of Iraq). In the current study, data presented that all the patients and healthy control subjects were non-smokers. Also, most of the volunteers from both patients and healthy control were from urban, all of them acquired a well-educated and they had a good work place, as shown in Table 1. The major variations between urban and rural regions are the differences in environments, pollution, social, psychic, genetic, food habits and others which are increasing dramatically in urban areas [16]. Therefore, our results cannot appear the actual status of the whole patients' groups in Iraq due to the low number of patients who attends in the diabetes and endocrine glands center in Al-Mawany teaching hospital and counts on the cooperatively of patients and their ready to engage in the present study as well. OPG plays a crucial function in atherosclerosis, arterial calcification, and vascular disease, it is highly expressed in the liver, kidney, bone marrow, and other tissues and produced by a variety of cell types including endothelial cells and smooth muscle cells; recent studies suggested that OPG may be a new marker for diabetic cardiovascular complications and atherosclerosis. Elevated concentrations of OPG have been reported in diabetic individuals and were independently associated with the diabetic microvascular complications [20]. Also, OPG has been reported to increase cell proliferation of human artery and vein endothelial cells, maybe through motivating phosphorylation of extracellular signal-regulated kinases1/2 and protein kinase B in these cells, which are like the effects of these growth factors such as fibroblast growth factor and vascular endothelial growth factor in the endothelial cells [21]. Clinical studies indicate that serum OPG

concentrations are related with coronary artery disease, vascular calcification, diabetic complications, and cardiovascular mortality [22]. This study demonstrated that OPG is significantly increased in type 2 diabetic patients with and without nephropathy as compared with control subjects. In addition, the study identified OPG as a significant marker of diabetic nephropathy. In the past, OPG has been noticed as an important inhibitor of bone resorption. However, in vivo studies have shown that OPG-deficient mice are recumbent to calcification of the aorta and nephritic arteries; and OPG can act as an existence factor for smooth muscle cells by motivating matrix metalloproteinase–9 activities [23]. There is also elevating clinical clue showing that increasing OPG concentration is associated with diabetic neuropathy, diabetic maculopathy and silent myocardial ischemia in type 2 diabetes mellitus patients [24]. Moreover, another study illustrates that OPG was increased in microalbuminuria and macroalbuminuria of type 2 diabetes mellitus patients as compared with normoalbuminuria of T2DM patients [25]. Taken together with the results of this study, these findings suggest that OPG might play a serious role in the pathogenesis of diabetic nephropathy. In an in vivo study, they found that OPG-treated mice showed a decreasing in function resulting in increased islet inflammatory cell infiltration, fibrosis, and apoptosis. They discovered that this OPG-induced remodelling of the islet structure was linked with increases in the expression of the renin-angiotensin system, which is known to be a large pathophysiologic response when diabetic nephropathy develops [26]. Recently, another study explained that serum OPG was elevated in type 2 diabetes mellitus patients without vascular complications as compared with normal healthy subjects [27]. This result marked that a higher serum OPG outruns the development of diabetic vascular complications. Considering those and our results, OPG appears to be a more sensitive marker in the diabetes state islet and may play a pivotal role in diabetic nephropathy. Some studies suggested that increased serum OPG levels have been interpreted as an insufficient compensatory self-defensive response to prevent further bone loss and the progression of atherosclerosis [28]. Insulin resistance (IR) can be known as a form of biological misinformation in the body in which the insulin hormone receptors on the cell membrane are not suitably responding to the insulin, thus the glucose in blood becomes unable to get into cells, which makes a hypoglycaemic reaction. This condition makes the pancreas produces high doses of insulin to attempt to get the glucose out of blood into cells, so decreased of the ability of insulin hormone to adjust and to signal changes the levels of glucose in the blood and possibly grows IR [16]. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was utilized as a substitute measure of insulin resistance in our study. Although HOMA-IR was not the gold standard for assessment of insulin sensitivity, it was a clinically useful index used in many studies [29]. Population-based studies for defining cut-off values of HOMA-IR for the diagnosis of insulin resistance have been conducted in different geographic areas. At present, there is no national survey data about the cut-off values of HOMA-IR in Iraqi population. Thus, we defined HOMA-IR index >1.8 as a cut-off point to indicate increased insulin resistance according to previous studies [31]. Although the cut-off point 1.8 may not be suitable for the Iraqi population, it can provide a useful reference point for our study when there is no available data for the Iraqi population. Previous reports had reported similar results about the association between serum OPG levels and HOMA-IR in subjects with type 2 diabetes mellitus [20]. Our findings are in line with a recent report, the authors found serum OPG levels were significantly associated with HOMA-IR in Chinese population, and serum OPG levels were significantly higher in subjects with impaired glucose regulation and diabetes than in those with normal glucose regulation [14]. Thus, some studies suggest that increased OPG creating represents a precocious event in the natural history of diabetes, probably participating to diabetes-associated vascular endothelial cell dysfunction [31]. In our study, serum OPG levels displayed significant association with IR, but the mechanisms implied the relation are currently ambiguous. It is assumed that inflammation may bind OPG to IR. IR is a hallmark of T2DM and considered as a chronic low-grade systemic inflammation [32]. It has been illustrated that OPG was positively correlated with inflammatory signs and played a causative function in the pathogenesis of inflammation [33].

OPG/RANK/RANKL system is considered to be related with the organizing of inflammatory and immune responses and straight shared to the organizing of proinflammatory cytokine generation in macrophages [34]. Moreover, it is recognized that the OPG/RANK/RANKL system could operate the NF-κB pathway and its downstream players, which are nearly associated to the pathogenesis of insulin resistance [35; 36]. Therefore, OPG perhaps have a function in IR through NF-κB pathway. Recently, proof has marked that the OPG/RANK/RANKL system may have a prospective role in the pathogenesis of diabetes; obstructing this pathway enhanced hepatic insulin resistance and banned the evolution of diabetes mellitus [37]. It has been realized that the OPG is a cytokine that raised the mineral density and volume of bone tissue by lowering the number of active osteoclasts. Serum OPG levels were significantly related with GFR, which is the marker of renal function, and these findings may be clarified as follows: OPG was highly expressed in the kidney, and there were significant relations between high OPG serum levels and kidney dysfunctions [20]. The serum OPG was significantly elevated in patients with chronic kidney disease. Recent proofs proposed that inflammation, secondary hyperparathyroidism, disorder of bone metabolism, vascular calcifications, and atherosclerosis may play crucial functions in this operation [38]. In our work, levels of selenium (Se) were found to be high significantly lowered in T2DM patients with or without DN when compared to healthy control group. Selenium is the master element in glutathione peroxidase "GPx" (an active enzyme against oxidative stress) that diminishing the formation of free radicals in the body. This lack in serum of selenium levels could participate to oxidative stress and low selenium level has been shown to decrease insulin secretion and raised insulin resistance in some empirical models, thereby possibly occupying a causal function in the progress and pathogenesis of T2DM [17]. Elevated oxidative stress and glycosylation play a major pathogenic action in diabetic endothelial cell dysfunction. Several experimental investigations examined antioxidant status in DN [39]. Plasma GPx level was decreased in T2DM patients with DN than those without DN or control subjects [40]. In another study, they found that Se supplementations were efficient to boost cellular GPx action in patients with various stages of chronic kidney disease. Nephropathy was linked to proximal tubule injury and loss of glomerular filtration barrier integrity and it is a sign of endothelial cell dysfunction due to abundant factors, including inflammation, insulin resistance, and oxidative stress [41].

Zinc (Zn) is an important trace element with antioxidant properties. Furthermore, zinc deficiency has been documented in patients with CKD and also in patients with longstanding diabetes. It may play a crucial role in the development of both T1DM and T2DM. Some previous studies were suggested that serum zinc level is associated with T2D, and loss-of-function mutations in zinc transporter-8 gene protect against T2D [42]. Our study indicated that a Zn level in diabetic patients with and without nephropathy was high statistically lower than the control group. The possible elucidation of the present results comes as following: in the mammalian pancreas, Zn is fundamental for the proper processing, storage, secretion, and action of insulin in beta cells. Insulin is stored inside secretory vesicles or granules, where two Zn^{+2} ions coordinate to six insulin monomers to form the hexameric structure on which mushy insulin crystals are established [43]. It is also known that like, most other chronic disorders, diabetes increases the excretion of more minerals such as Zn in urine than non-diabetics or may be decrease gastrointestinal absorption of Zn. Also, hyperglycemia in diabetes is usually associated with hyperzincuria, which is of renal origin, and increased urinary loss of Zn^{+2} and decreases of its concentration in total body Zn^{+2} [17]. Renal tubular defect in handling zinc and glucose-induced, osmotic diuresis are other possibilities. Zn deficiency is associated with metabolic disturbances including impaired glucose tolerance, insulin degradation, and reduced pancreatic insulin content. Furthermore, Zn may improve glycemia, and a restored Zn status in patients with type 2 diabetes may counteract the deleterious effects of oxidative stress, helping to prevent complications from beneficial antioxidant effects in persons with type 2 diabetes. On the other hand, it has been reported that Zn may have

special importance considering the deleterious consequences of oxidative stress in persons with diabetes [18]. Also, this trace element has antioxidant properties; thus, it can stabilize macromolecules against radical induced oxidation. Moreover, it plays a key role in the synthesis, secretion and action of insulin in both physiological and pathological situations. In addition, recent studies have highlighted Zn's dynamic role as a "cellular second messenger" in the control of insulin signalling and glucose homeostasis [44]. Finally, there was some evidence which shows that Zn acts as an antioxidant. Under Zn deficiency, free radicals are activated due to an impaired antioxidant defence system and imbalances in the production of free radicals. High oxidative stress conditions created this situation are involved in the pathogenesis of diabetes and its related complications [17]. In addition, Magnesium (Mg) is one the most abundant cation in the human body and plays a key role in many fundamental biological processes, including energy metabolism and DNA synthesis. Mg deficiency has been shown to cause endothelial cell dysfunction, inflammation, and oxidative stress, which are major contributors to atherosclerosis [45]. Some epidemiologic studies have reported associations between low Mg intake or serum Mg level and hypertension, coronary artery disease, and ischemic stroke [46]. Mg and type 2 diabetes have a close relationship. Approximately one-third of patients with type 2 diabetes have hypomagnesemia, mainly caused by enhanced renal excretion. Mg deficiency is associated with poor glycemic control, and Mg supplementation improves insulin sensitivity [47]. Moreover, there is substantial evidence of associations between hypomagnesemia and various complications of type 2 diabetes, including neuropathy, retinopathy, foot ulcers, and nephropathy [48].

In our study, the serum value of Magnesium "Mg" showed high statistically significant decreases in diabetic patients with and without NP when compared to healthy subjects. Moreover, Mg is necessary for several enzymes that play an important role in glucose metabolism. The hypomagnesaemia in diabetic nephropathy might be due to poor dietary intake, impaired absorption of Mg, increased urinary loss due to hyperglycemia, osmotic diuresis, defective Mg reabsorption from renal tubules and loss of plasma protein bound Mg. Mg depletion is said to reduce insulin sensitivity, thereby increasing the risk of secondary complications. Hyperglycemia leads to decreased cellular Mg levels. Hypomagnesaemia leads to collagen and ADP-induced platelet agreeability and decreased function of Mg dependent enzymes, kinases and oxidative stress [18]. Hypomagnesaemia can increase platelet reactivity, increase vascular and adrenal responses to angiotensin II, enhance thromboxane A2 (TXA2) release, and lead to organ damage from free radicals [17]. The intracellular depletion of myo-inositol due to disruption of its paracellular transport mechanisms is a major factor in the development of diabetes complications in magnesium deficiency. Therefore, magnesium deficiency has specific pathogenic significance in diabetic nephropathy. Magnesium chloride supplementation lowers HbA1c, improves the Insulin sensitivity in type 2 diabetics and higher magnesium intake is associated with lower risk of diabetes in the general population. Insulin resistance probably explains the relation between diabetic nephropathy and hypomagnesemia; magnesium deficiency reduces tyrosine kinase activity, post-receptor activity, and insulin-dependent glucose uptake, thereby leading to insulin resistance. In addition, increased intracellular calcium in magnesium deficiency interrupts skeletal muscle and adipocyte response to Insulin. On the other hand, Insulin deficiency and resistance lead to reduced tubular reabsorption of magnesium and ensuing hypomagnesemia favor the onset and progression of diabetic microangiopathy, via a reduction in activity of Na+/K+ ATPase pump [49]. A Recent study concluded that hypomagnesemia independently predicts the progression to end-stage renal disease in patients with advanced diabetic nephropathy [50].

5. Conclusion

From this study it is concluded that increased serum OPG and decreased trace elements (Se, Zn and Mg) levels are strongly associated with BMI, insulin resistance and physical activity which can be used as a

biomarker of renal dysfunction in diabetic nephropathy thus decreasing the mortality and morbidity. Hence, taking into consideration the combination of obesity, insulin resistance and NIDDM may demonstrated excellent indicator for early-stage diabetic nephropathy in patients with type 2 diabetes. However, further studies using larger sample sizes should be performed to establish the diagnostic value of other biomarkers for detection of early-stage diabetic nephropathy.

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