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Eleveted Vaspin Level S in Diabetic Nephropathy

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ABSTRACT

Background:

Chr0nic kidney disease "CKD" patients have insulin secreti0n dis0rders and resistance to insulin effects, that is resp0nsible for the devel0pment of cardi0vascular proceedings. Vaspin is an adip0cyt0kine that regulates gluc0se and lipid metab0lism. We aimed t0 determine the serum vaspin levels and its relati0nship with insulin resistance in "CKD"patient's.

Objective: To assessvaspin levels in diabetic nephropathy and find the possibility of use vaspin in diagnosis (DN).

Patients and Meth0ds: In this studygr0ups,serum vaspin levels,and r0utine bl00d tests were measured. C0nduct with 120 patients and 60control.The patients divided int0 three gr0ups: (n=60) diabetic patients , (n=60) diabetic nephropathy, and number of control (n=60). The serum vaspin levels were tested by the enzyme-linked immune s0rbent assay(ELISA).

Result: The results were compared with healthy groups. In this present study, observed that the mean age of patients with diabetic patintes was (45.05±13.09years,).with diabetic nephropathy(52.47±11.15years), and in the control (43.01±12.77 years).The serum vaspin were 0bserved significantly high in the diabetic patient and diabetic nephropathy gr0ups in contrast with the control gr0ups.The female predominance in three groups, female to male ratio was (2:1), where18(30.00%) of patients were male and 42(70.00%)were female in diabetic groups. where23(38.30%) of patients were male and 42(70.00%)were 37(61.70%)female in diabetic nephropathy groups .The mean of Vaspin in diabetic patient groups and in diabetic nephropathy groups and control groups are 464.10±198.01' 563.98±100.12,and140.96±33.36 pg/ml. vaspinwas increase in patients with diabetic patients. The serum vaspin level was negative c0rrelated with the fasting plasma gluc0se with0ut statistical significance.

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Conclusi0n:"Diabetic nephr0pathy" patients presented a significant increased levels of serum Vaspin when compared to levels to control, serum Vaspin are protective roles.We c0nsider that the rise in the serum vaspin level is a c0nsequence of the reduced renal excreti0n in the (CKD) and increases in resp0nse t0 insulin resistance.

Keywords: serum vaspin, ,biomarker, diabetic nephropathy.

Introduction:

Diabetic nephropathy (DN) or diabetic glomerular sclerosis pathologically **(Yang *et al* .,2015)** . 0ne of the most feared chr0nic micr0vascular c0mplications is diabetic nephr0pathy (DN), New termin0l0gy refer to kidney illness attributable to diabetes is being recent guidelines (National Kidney Foundation (**NKF-KDOQI .,2007).**So, the term "diabetic nephr0pathy" should be exchanged by diabetic kidney disease introduced in (DKD), a long-term highly prevalent main micr0vascular c0mplicati0n defined as structural, functi0nal, and clinical abn0rmalities of the kidneys that are produced by diabetes **(Mora-Fernandez *et al* .,2014).**currently the leading reason of end--stage renal disease (ESRD) in the Western world. Strikingly, 40-45% of patients with type-1 diabetes (T1D) develop DN and reach ESRD or passaway before its onset. Moreover, clinicians face a "30%"prevalence of patients with type-2 diabetes (T2DM) and DN with "45%" of patients currently on dialysis having a primary analysis 0f diabetes, a p0pulati0n similarly at high hazard 0f devel0ping cardiovasculardisease **(Gnudi*et al* .,2016).**An primary indication of DN is an increased quantity of urinary protein, manifested by (albuminuria), which ass0ciates with, and can predict, the progression of renal damage. Albuminuria arises fr0m defects in the permeabilityof the gl0merular filtration barrier consisting of glomerular endothelial cells{GECs} separated from specialized epithelia, called p0d0cytes, by the glomerularbasement membrane (GBM) **(Gnudi*et al* .,2016).**Adip0setissue is the biggest s0urce of energy in the b0dy. This energy is st0red in the f0rm 0f triglycerides, which can quickly g0 thr0ugh circulation as fatty acids in case of hunger or when needed.Vaspin(visceral adip0se tissue-derived serpin) is a n0vel adip0cyt0kine that regulates gluc0se and lipid metab0lism**.(Li Q *et al*.,2008)** It is a member of the serine pr0tease inhibit0r family and is secreted in 0tsuka L0ng–Evans T0kushima Fatty (0LETF) rats in case of 0besity and peak insulin plasma c0ncentrati0ns from the visceral adip0se tissue. **(Hida K *et al* .,2005)**Vaspin is c0nsidered a

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n0vel bi0marker that is p0tentially effective against 0besity and reduced insulin t0lerance, and it is released as an ant pr0tease fact0r in visceral adip0se tissue; clinically w0rsening diabetes and weight l0ss decrease vaspin expressi0n.

Patients and Methods:

This study was carry out during the period from November 2019 until April 2020.One hundred and twenty(120) patients have type 2DM ,with, Normoalbuminuria,and albumin urea andThis subgroup included patients with microalbuminuria, ACR = 30-300 mg/g creatinine. in AL-Aziziya general hospital in waist seeking for medical help regarding their recently developed symptoms. **Inclusion criteria:** Included studies of adult humans with type2 diabetes nephropathy Type 2 diabetes mellitus with a hemoglobin A1c (HbA1c) greater than or equal to (>=) 6.5 percent (%). withalbumin creatinine ratio.

Exclusion criteria: Polycystic ovary , obesity, osteoarthritis, , thyroid disease, acne vulgaris patients, coronary artery disease, ,liver disease, renal disease that due to other thandiabetes **.**

Seven milliliters (7 ml) blood of ven0us have be taken from aseptic area of patients and control. Ven0us bl00d samples were 0btained fr0m allpatients f0ll0wing a “12 h” fasting f0r bi0chemical analyses separate serum by centrifugati0n at4000 rpm for ten min. and splatted into three parts: Aliquot of serum was transported into 1.5 ml Eppend0rf tube, this part was use for assay (T0tal Ch0lester0l TC, Triglyceride TG, High Density Lipopr0tein-Ch0lesterol HDL-C and bl00d sugar). Use EDTA for testing HbA1c .bl00d taken f0r the serum vaspin level was st0red in the tube c0ntaining apportioning f0r ten min and then centrifuged at 4.000 rpm for ten min. The 0btained serum was stored at −20 °C in the deep freezerThe collected samples were solved in the laboratory environment and analyzed with Human (VASPIN) ELISA Kit Catalog No: MBS2506005acc0rding to the manufacturer’s instructions in an ELISA readerHS(Germany).The BMI values 0f the patients were rec0rded as kg/ m2.

serum lipid pr0file test (T0tal cholesterol TC, triglyceride TG, High Density Lipoprotein (HDL), L0w Density Lipoprotein LDL, Very Low Density Lipoprotein (LDL) Fasting blood sugar FBS by fully automated . The statistical analysis: case control. (UACR) Mid-stream random spot

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urine samples were also collected from study groups diabetic nephropathy(60 patints) into disposable screw cup containers for estimation albumin to creatinine ratio. by fully automated.

Result: The cases were divided int0 three gr0ups as the c0ntr0l and the diabetic,diabetic nephropathy groups. When the gr0ups were c0mpared in terms of dem0graphic characteristics,FBG ,Hb A1c ,Serum lipid profiles were appearing to be significantly increase all t0gether in b0th groups of diabetic and diabetic nephropathy patients . apart from the serum HDL-C which found to be decrease serum levels in contrast with control group, revealed a highly significant increase in Vaspin levels in (diabetic ,diabetic nephropathy) group of patients in compare with group control statistically significant difference ($p<0.001$) was found in (Table-I) (Figure-1) show the increase serum Vaspin levels 0f the three studied gr0ups.

In (Table-I).the number of female in the group diabetic patients was42(70.00%) and male was 18(30.00%) .the number of female in the group diabetic nephropathypatients was 37(61.70%) and male was 23(38.30%).The number of female in the control group was 40(66.70%) and number of male were 20(33.30%), The observed frequencies in female group was more than the group of male in ratio (2:1) and there is statistically no a significant differenc between the frequency 0f the patients in male and female gr0ups c0mpared to c0ntrol gr0up.(P=0.62).BMI also show no a significant difference($p= 0.30$)between the three groups .in (Table-2)vaspin levels correlation negative with BMI ($r=-0.34$) ($p=0.01$) , vaspin levels no significant c0rrelation positive ($r= 0.97$) ($p=0.00$) with diabetic patient groups in (Table-3) vaspin levels was shown no significant correlation negative with (uACR)($r=-0.30$) ($p=0.01$) in diabetic nephropathy groups.

Table (1): The biomarkers levels.and biochemical variables among the three studies groups.

Parameter	Control (n=60) mean±SE	Diabetic nephropathy with normo albumin urea group (n=60) mean±SE	Diabetic nephropathy with albumin uria(micro, macro albumin urea) patients group (n=60) mean±SE	ANOVA P value

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Age (years)	43.01±12.77[a]	45.05±13.09[a]	52.47±11.15[b]	0.001
Gender Male Female	20(33.30%) 40(66.70%)	18(30.00%) 42(70.00%)	23(38.30%) 37(61.70%)	0.62
Weight (kg)	59.42±3.14[a]	59.42±3.14[a]	60.00±2.87[a]	0.49
BMI (Kg/m^2)	21.74±3.21[a]	22.12±1.41[a]	22.34±1.31[a]	0.30
FPG (mg/dl)	91.16±12.58[a]	254.67±85.25[b]	214.91±47.87[c]	0.001
HbA1c (%)	5.17±0.62[a]	7.53±1.20[b]	7.36±0.98[b]	0.001
S. TC (mg/dl)	165.55±10.07[a]	212.83±42.55[b]	214.27±48.61[b]	0.001
S. TG (mg/dl)	131.28±11.01[a]	201.62±40.18[b]	198.64±42.97[b]	0.001
LDL-C (mg/dl)	78.67±10.98[a]	137.41±43.30[b]	138.50±48.19[b]	0.001
HDL-C (mg/dl)	54.18±4.60[a]	36.65±5.37[b]	36.03±6.20[b]	0.001
VLDL-C (mg/dl)	33.03±2.17[a]	40.32±8.03[b]	39.72±8.59[b]	0.001
ACR (mg/mmol)	—	—	247.15±55.01	—
S. Vaspin (pg/mL)	140.96±33.36[a]	464.10±198.01[b]	563.98±100.12[b]	0.001

Different small letters den0te significant differences.

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Similar small letters den0te n0n-significant differences.

Table 2. The person correlation analysis of diabetic nephropathy with normo albumin urea patients group.

	Age (years)	Weight (kg)	BMI (Kg/m2)	FBG (mg/dl)	HbA1c (%)	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	S.Vaspin (ng/ml)	GFR(ml/min/1.73 m2	ACR (mg/mmol)
Age (years)	r=1	0.18 0.16	-0.01 0.90	0.15 0.25	0.28 0.03	0.14 0.28	-0.05 0.65	0.12 0.34	-0.18 0.15	-0.05 0.65	0.00 0.99	-.472 .000	-.050 .709
Weight (kg)	r=0.18 *P*=0.16	1	0.35 0.01	-0.00 0.99	-0.07 0.56	0.10 0.41	-0.18 0.15	0.15 0.24	-0.06 0.63	-0.18 0.15	0.02 0.84	-.026 .845	.086 .515
BMI (Kg/m^2)	r=-0.01 *P*=0.90	0.35 0.01	1	0.07 0.55	-0.02 0.87	0.02 0.84	-0.04 0.73	0.04 0.73	0.06 0.61	-0.04 0.73	-0.34 0.01	.200 .129	-.107 .420
FBG (mg/dl)	r=0.15 *P*=0.25	-0.00 0.99	0.07 0.55	1	0.45 0.00	0.55 0.00	0.42 0.00	0.47 0.00	-0.13 0.31	0.42 0.00	-0.10 0.44	.013 .925	.091 .494
HbA1c (%)	r=0.28 *P*=0.03	-0.07 0.56	-0.02 0.87	0.45 0.00	1	0.19 0.13	0.15 0.25	0.17 0.19	-0.17 0.17	0.15 0.25	-0.08 0.53	-.193 144	-.023 .863
TC (mg/dl)	r=0.14 *P*=0.2	0.10 0.41	0.02 0.84	0.55 0.00	0.19 0.13	1	0.34 0.01	0.95 0.00	-0.31	0.34 0.01	-0.09	-.037	-.095 .476

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	8								0.01		0.47	.782	
TG (mg/dl)	r=-0.05 *P*=0.65	-0.18 0.15	-0.04 0.73	0.42 0.00	0.15 0.25	0.34 0.01	1	0.24 0.06	-0.17 0.18	1.00 0.00	-0.22 0.08	-.115 .384	-.233 .076
LDL-C (mg/dl)	r=0.12 *P*=0.34	0.15 0.24	0.04 0.73	0.47 0.00	0.17 0.19	0.95 0.00	0.24 0.06	1	-0.39 0.00	0.24 0.06	-0.08 0.53	-.017 .901	-.045 .735
HDL-C (mg/dl)	r=-0.18 *P*=0.15	-0.06 0.63	0.06 0.61	-0.13 0.31	-0.17 0.17	-0.31 0.01	-0.17 0.18	-0.39 0.00	1	-0.17 0.18	0.12 0.36	-.109 .412	.044 .741
VLDL-C (mg/dl)	r=-0.05 *P*=0.65	-0.18 0.15	-0.04 0.73	0.42 0.00	0.15 0.25	0.34 0.01	1.00 0.00	0.24 0.06	-0.17 0.18	1	-0.22 .0.08	-.115 .384	-.233 .076
S.Vaspin (pg/mL)	r=0.00 *P*=0.99	0.02 0.84	-0.34 0.01	-0.10 0.44	-0.08 0.53	-0.09 0.47	-0.22 0.08	-0.08 0.53	0.12 0.36	-0.22 0.08	1	.022 .868	.051 .703
GFR ml/min/1.73 m2	r=-472 P=.000	-026 845	.200 .129	.013 .925	-.193 .144	-.037 .782	-.115 .384	-.017 .901	-.109 .412	-.115 .384	.022 .868	1	.213 .105
ACR (mg/mmol)	r=-050 P=709	.086 .515	-.107 .420	.091 .494	-.023 .863	-.095 .476	-.233 .076	-.045 .735	.044 .741	-.233 .076	.051 .703	.213 .105	1

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Table -3..The person correlation analysis of diabetic nephropathy with albumin urea micro and macro albumin urea patients group.

	Age (years)	Weight (kg)	BMI (Kg/m2)	FBG (mg/dl)	HbA1c (%)	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	S.Vaspin (ng/ml)	ACR (mg/mmol)	GFR(ml/min/1.73m2
Age (years)	r=1	-0.15 0.24	0.04 0.74	-0.18 0.15	-0.13 0.31	-0.11 0.38	-0.13 0.31	-0.12 0.33	0.27 0.03	-0.13 0.31	0.04 0.73	0.11 0.40	.074 .576
Weight (kg)	r=-0.15 *P*=0.24	1	0.25 0.05	-0.26 0.04	-0.17 0.18	0.13 0.32	0.15 0.22	0.10 0.41	-0.04 0.75	0.15 0.22	0.00 0.99	0.05 0.69	.058 .660
BMI (Kg/m^2)	r=0.04 *P*=0.74	0.25 0.05	1	-0.17 0.19	-0.15 0.23	-0.01 0.89	-0.27 0.03	0.02 0.87	0.07 0.57	-0.27 0.03	-0.03 0.80	-0.06 0.60	.005 .973
FBG (mg/dl)	r=-0.18 *P*=0.15	-0.26 0.04	-0.17 0.19	1	0.68 0.00	0.00 0.99	0.11 0.39	-0.02 0.88	-0.00 0.98	0.11 0.39	0.01 0.91	0.05 0.67	-.085 .520
HbA1c (%)	r=-0.13 *P*=0.31	-0.17 0.18	-0.15 0.23	0.68 0.00	1	-0.04 0.71	0.14 0.27	-0.09 0.46	0.17 0.17	0.14 0.27	0.00 0.98	0.06 0.65	-.115 .387
TC (mg/dl)	r=-0.11 *P*=0.38	0.13 0.32	-0.01 0.89	0.00 0.99	-0.04 0.71	1	0.39 0.00	0.98 0.00	-0.34	0.39 0.00	0.01 0.8	-0.25	-.049

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									0.01		8	0.05	.711
TG (mg/dl)	r=-0.13 *P*=0.31	0.15 0.22	-0.27 0.03	0.11 0.39	0.14 0.27	0.39 0.00	1	0.25 0.05	-0.30 0.02	1.00 0.00	0.11 0.39	-0.25 0.05	-.021 .877
LDL-C (mg/dl)	r=-0.12 *P*=0.33	0.10 0.41	0.02 0.87	-0.02 0.88	-0.09 0.46	0.98 0.00	0.25 0.05	1	-0.42 0.00	0.25 0.05	-0.00 0.96	-0.21 0.10	-.044 .741
HDL-C (mg/dl)	r=0.27 *P*=0.03	-0.04 0.75	0.07 0.57	-0.00 0.98	0.17 0.17	-0.34 0.01	-0.30 0.02	-0.42 0.00	1	-0.30 0.02	0.04 0.76	-0.01 0.93	-.016 .906
VLDL-C (mg/dl)	r=-0.13 *P*=0.31	0.15 0.22	-0.27 0.03	0.11 0.39	0.14 0.27	0.39 0.00	1.00 0.00	0.25 0.05	-0.30 0.02	1	0.11 0.39	-0.25 0.05	-.021 .877
S.Vaspin (pg/mL)	r=0.04 *P*=0.73	0.00 0.99	-0.03 0.80	0.01 0.91	0.00 0.98	0.01 0.88	0.11 0.39	-0.00 0.96	0.04 0.76	0.11 0.39	1	-0.30 0.01	.060 .649
ACR (mg/mmol)	r=0.11 *P*=0.40	0.05 0.69	-0.06 0.60	0.05 0.67	0.06 0.65	-0.25 0.05	-0.25 0.05	-0.21 0.10	-0.01 0.93	-0.25 0.05	-0.30 0.01	-.036 .789	1

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GFR ml/min/1.73 m2	r=.074 p=.576	.058 .660	.005 .973	-.085 .520	-.115 .387	-.049 .711	-.021 .877	-.044 .741	-.016 .906	-.021 .877	.060 .649	1	-.036 .789

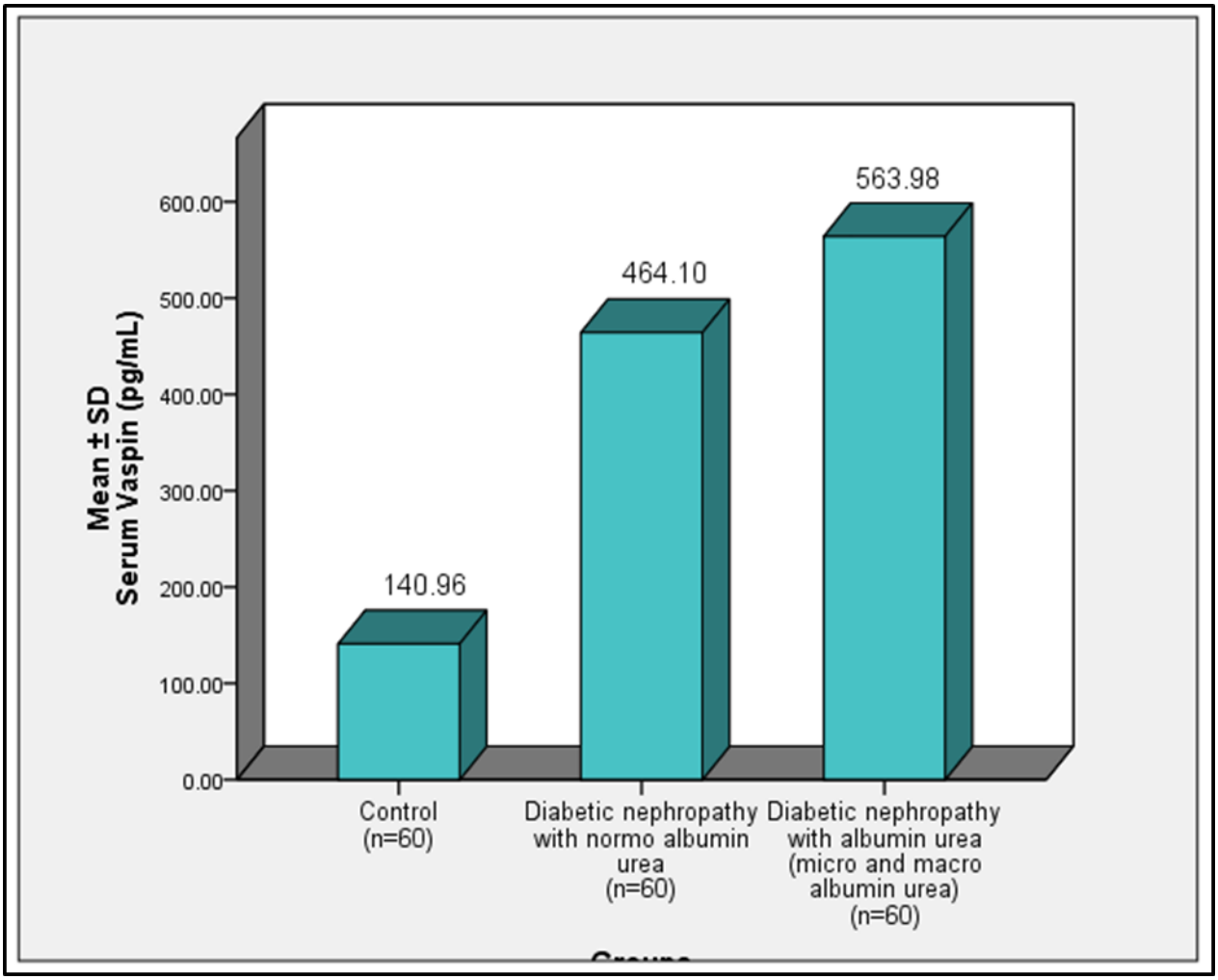

Figure 1. The serum Vaspin levels of the three studied groups.

DISCUSSI0N:

Vaspine is an adip0cyt0kine that plays a regulat0ry r0le in gluc0se and lipid metab0lism**.(Li Q *et al* ., 2008)**.T2DM is categorized by impaired insulin secreti0n and variable degrees of insulin resistance. **Youn*et al***. also f0und that patients with 0besity 0r abn0rmal insulin sensitivity had raised serum vaspin levels **(Youn*et al* .,2008)** .(Table -1). The average age of patients with diabetic nephropathy. significant increase (p=0.001) in at different ages, comparing with control

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groups. Which was agreed with **piotie study (2015),**there was n0 significant difference of b0dy mass index (BMI) (p value =0.30) for three groups this study agreement with study (**Erkan C *et al*., 2003).**a significant increase (P<0.001) in fasting bl00d gluc0se (FBG) , HBA1c between diabetic patients and diabetic nephr0pathy . Many studies were c0nsistent with the results of this study,**(Hintsa *et al*.,2017) .**the mean 0f parameters 0f lipid profile (cholesterol, triglycerides, LDL, VLDL) in type 2-DM was significant higher(P=0.001) than its counterpart in healthy controls**.** Only serum high density lipoprotein (HDL) showed decrease in level of type 2-DM this study agreement with previous study **(Samantha *et al* .,2012)** in the diabetic nephropathy show that there is a significant increase(p<0.001) the mean of parameters of lipid profile and increased breakdown of HDL**(Kawanami*et al* .,2016).** In table one show which found that serum vaspin was a highly significant (p=0.001)(464.10±198.01, 563.98±100.12) comparing with control group(140.96±33.36). increase in two studied groups(type 2 DM,DN) this study agreement with **Rai *et al* .,(Rai *et al* ., 2013).(Gulcelik NE e*t al* .,2009)** higher vaspin levels were f0und in the patients with nephr0pathyThe mean serum (cholesterol, TG, LDL, VLDL) level was higher significant in diabetic nephropathy as compared with the control (P<0.001). but HDL was low (p<0.05) in diabetic nephropathy as compared the with control. this study agreement with **Tsimihodimos*et al*. (Tsimihodimos*et al* ., 2011)** The presented study is the first 0ne that expl0red the c0rrelation 0f the serum vaspin level and the DNP stages. In this study, the circulating serum vaspin level was found to be significantly negative correlated with body mass index(r= -0.34) (p=0.01). However, other study found no correlation between serum vaspin level and body mass index**(Seeger *et al* ., 2008)(Aust*et al* ., 2009).** But **Youn*et al*. 2008(Youn*et al* ., 2008).** found the serum vaspin level p0sitively c0rrelated with obesity, and the visceral vaspin p0sitively c0rrelated with body mass index which indicate that the serum vaspin induced by obesity, mainly because the vaspin is an adipocytokine secreted from adipocytes **(Klöting*et al* .,2006).**a negative correlation between circulating serum vaspin and HbA1c% in all type2 diabetic patients. Other study reported that lower vaspin level was correlated with HbA1c above 7**(Seeger *etal* ., 2008).** in this presented study there was aweak positive c0rrelation as f0und between serum vaspin and HDL but no correlations between serum vaspin and LDL or triglyceride.**Jian*et al*** showed that the serumvaspin c0ncentration did not c0rrelate with triglyceride in type 2 diabetic patients. So the vaspin may have less effect 0n lipid metab0lism **(Jian *et al* .,2014).**(Table-3) show negative c0rrelation vaspin with (uACR) in

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diabetic nephropathy groups (r=-0.30) (p=0.01). this study present disagreement with the study of ***Inoueet al*** (***Inoueet al*.,2012) .**thisobtainable study is the first 0ne that expl0red the c0rrelation 0f the serum vaspin level with albumin creatinine ratio in the DNP stages. study of diabetic nephropathy with normo albumin urea by **Gulcelik et al.,(Gulcelik NE et al.,2009)** higher vaspin levels were found in the patients with diabetic nephropathy with albumin urea (micro and macro) relative to the patients with no nephropathy the results of this study could be explained by two factors: in our study, all the patients are diabetic, and there are no hemodialysis patients. Since vaspin is a small protein(50kD), it could be freely filtered by the kidneys.

Conclusion:

Vaspin, an important role in protective effects on "type 2 "DM and diabetic nephr0pathy and measurement 0f Vaspin in patients. with diabetic patients may add in diagnosis and to prevent complication of patients with diabetic nephropathy .

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