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Correlation of retinol binding protein 4 with insulin resistance and genetic study for endothelial nitric oxide synthase G894T and glucose transport -1 in diabetes mellitus type 2 nephropathy

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Abstract : Diabetes mellitus (DM) is a metabolic disease involving in metabolism disorder. Diabetic nephropathy (DN) is the significant complication of diabetes, which is at the present time the major cause of chronic renal failure. Retinol binding protein 4(RBP)for is a plasma protein that secreted primary from liver and adipose tissues. Aim of the study to evaluate the RBP and related to genes of endothelial nitric oxide synthase G894T and glucose transport -1 in diabetes mellitus type 2 nephropathy. This study was included 160 subjects{80 control (C),80 patients}.The results show: There is significant increase in the fasting glucose, HbA_{1c}, fasting insulin level, insulin resistance, total cholesterol TC, TG, LDL-c and VLDL-c , Non HDL-c and RBP4 concentration in groups(M=Male and ,F=Female ,$p<0.001$), while concentration of serum HDL-c was found decrease significantly. There is significant positive correlation between RBP4 and insulin resistance in patients groups. According to the results of genotyping, XbaI polymorphism was identified as homologous genotype for patients:XbaI (-/-) 12 (30%) in the group M and 14 (35%) in the group F. In control groups 3(7.5%) in the group MC and in the group FC 2(5%), while individuals has heterozygous genotype XbaI (-/+) 7 (17.5%) in the group M, 10 (25%) in the group F, 10 (25%) in group MC and in the group FC 8(20%).A significant relationship between the of tenness of XbaI (-/-) variant in group M in compared with group MC an odds ratio = 5.14 and confidence interval at 95% level of (1.36 – 19.4), and in group F in compared with group FC an odds ratio = 13.12 and confidence interval at 95% of (2.64 – 65.07).

Also the results shown for the G864T polymorphism for eNOS gene was identified as homologous genotype TT in group M 7 (17.5%), in Group F 9 (22.5%), in group MC 8 (20%) and in group FC 10 (25%), while individuals has heterozygous genotype G/T in group M 21 (52.5%), in Group F 20 (50%), in group MC 6 (15%) and in group FC 7 (17.5%).A significant frequency of GT variant in group M compared with group MC with odds ratio = 7.58 and confidence interval at 95% level of (2.43 – 23.62), and in patients of group F compared with group FC with odds ratio = 7.27 and confidence interval at 95% of (2.4 - 22.02).The conclusion from this study, it is found that RBP4 is associated in causing insulin resistance and lipid abnormalities. The high levels of non HDL-c in diabetic patient contribute in progression of diabetic nephropathy. The GLUT1 polymorphism in diabetic type 2 patients specially patients

with XbaI (-/-) and eNOS gene polymorphism G/T allele have a role in progression to diabetic nephropathy.
Key words :retinol binding protein4, endothelial nitric oxide synthase G894T gene, glucose transport 1gene , diabetes mellitus , genotype ,diabetic nephropathy.

Introduction

Diabetes mellitus (DM) is the metabolic disease it is expressed precisely by persistent hyperglycemia, resulting from defects in insulin secretion, insulin action or summation of both, imperfect secretion and wrong action[1]. DM is sorted in accord with the pathogenic manner that arrives at hyperglycemia[2].Etiologically, classification of DM .Type1 diabetes, type 2 diabetes, immune-mediated Idiopathic and other specific types[3].Type 2 diabetes mellitus (T2DM) is complicated takes place when impaired IR is accompanied by the failure to produce ample amount of β-cell insulin[4].

Diabetic nephropathy (DN) is the critical entanglement of DM, which is right now the significant reason for chronic renal failure [5]. Physiologically, IR is a state of relationship in which cells fail to respond to the regular actions of insulin. When body synthesis insulin under insulin resistance situations, the cellsin a body are resistant to the insulin and are unableto utilize it efficiently, leading to high blood sugar[6]. Retinol binding protein (RBP4) is a one of,proteins, belongto the lipocalin family, which are transport of tiny hydrophobic molecules. The RBP4 gene located on chromosome10 (10q23–q24) close to the region that has been. linked to increased fasting glucose levels[7].Glucose influx in renal cells is modulated by GLUT1, which is surface receptor of inhabitant renal cells. Induction of overexpression ofGLUT1 mRNA and overproduction of GLUT-1 protein in mesangial cells is due to high concentrations of glucose [8]. The nitric oxide (NO) system be made up of three different isoforms of nitric oxide synthase (NOS), three distinct genes encoded them, including neuronal nitric oxide synthase(nNOS). Vascular endothelial impaired function resulting from impaired activity of NOS in the endothelial cells ,plays crucial role in the pathogenesis of DN[9].The aim of the study to investigate the role RBP4 in diabetic nephropathy patients and to evaluation of gene level of GluT1and eNOS G894T polymorphism in diabetic patients to predict its relationship with nephropathy in future.

Subjects and methods

Samples collection

The study groups were included (160) persons of the diabetes mellitus type 2 nephropathy patients and control, the age of them between (35 – 58) years, which were divided into the four groups {80 patients :40 Male (M), 40 female (F), 80 control: 40 male (MC)and 40 female (FC)}.They were collected from Babylon Center for Diabetes and Endocrinology in Marjan Teaching Hospital in Babylon / Hila city.The age and gender of this group were coincided with age and gender of patientgroups .

Ethical Issues:

Depends on the consent of the scientific committee in the Babylon University / College of Medicine.

Anthropometric Measures

All groups are defined as overweight (BMI values between 25 - 29.9).

Blood samples collection:

Venous blood samples were drawn from patients and control groups in fasting status. Ten ml of blood were obtained; 8 ml drawn slowly into tubes containing separating gel and the serum were obtained for measurement of glucose, insulin and lipid profiles. 2ml from the blood put in tubes containing EDTA and was utilized in the genetic analysis.

Measurements of Parameters

Plasma glucose were measured in fasting state by using the glucose oxidase method (Biolabo). Plasma insulin and RBP4 were analyzed by sandwich ELISA method (Monobind/USA). Total cholesterol, HDL cholesterol, triglycerides were measured by enzymatic colorimetric method. LDL cholesterol levels were calculated by utilizing Friedewald method. Non HDL-c was calculated with the assistance of deducting HDL-C from total cholesterol. Non HDL – c = Total cholesterol – HDL cholesterol[10].

Glycated Hemoglobin

Insulin resistance was measured using HOMA-IR (Homeostasis Model Assessment – Insulin Resistance), Retinol binding protein 4 was analyzed via sandwich ELISA method.

Genotyping analysis

DNA Mini Kit used to purifying genomic DNA from thewhole blood[11,12]. Purity of DNA was assessed by nanodrop instrument and electrophoresis on 0.8% agarose gel. By the UV trans-illuminator the agarose gel was visualised[13-15], fig. (1)

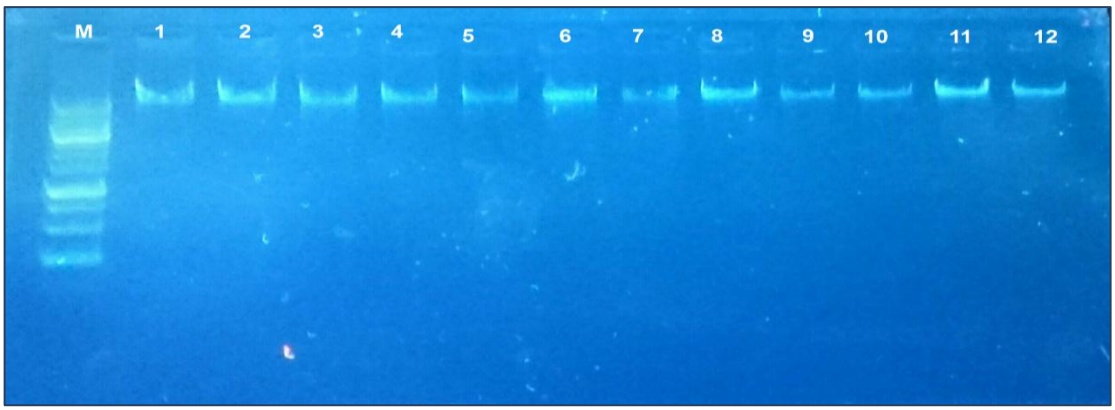

Fig.(1): DNA extraction from blood. Lane (M: DNA marker 1kb), lane (1-6patients): lane, (7-12controls).

Determination of glucose transport-1 (GLUT-1) genotyping:

According to theDaniel P.K.[16].

Determination of eNOS genotyping

Amplification G/T codon 894 of eNOS gene was done by using the following primer[17,18].

Statistical analysis

The results had been analyzed by utilizing SPSS version 22. The results was displayed as a mean and standard deviation (SD). Uninterrupted variables were used t- test. Genetic investigation was performed using Chi-square ($\chi 2$) test to estimate odd ratio (OR). P values <(* :0.05) was accounted significant and <(**: 0.001) accounted highly significant.

Results:

Age:

The results showed in table (1), no significant (P > 0.05) differences in age between diabetic groups and control groups.

Table(1): Mean and standard deviation of age in diabetic and control groups.

Parameter	Subjects	No.	Mean ±SD	P- value
Age (Years)	Group M	40	46.55 ±4.478	P = 0.575*
	Group F	40	47.23 ± 6.7	
	Group MC	40	45.45 ± 5.154	
	Group FC	40	46.15 ± 5.216	

Body Mass Index :

The results showed in table(2), there were no significant differences ($p > 0.05$) in body mass index between control groups and diabetic groups.

Table(2): Body mass index in control and patient groups.

Parameter	Subjects	No.	Mean ±SD	P-value
Body mass index (BMI)	Group M	40	26.9 ± 1.51	P = 0.957*
	Group F	40	27.1 ± 1.34	
	Group MC	40	27.6 ± 1.42	
	Group FC	40	26.7 ± 1.46	

Fasting Glucose, Glycated Hemoglobin, Fasting Insulin and Insulin Resistance (HOMA-IR) Measurements:

The results exhibit that glucose, insulin, HbA_{1c} and HOMA-IR were elevated significantly in patients groups compared with control groups ($P < 0.001$) , (table 3).

Table (3): Glucose,glycatedhemoglobin , insulin and HOMA-IR in in type 2 diabetic groups and control groups.

Parameter	Patients		Control		P -value
		Mean ± SD		Mean ± SD	
Glucose (mmol/L)	Group M	11.493 ±4.15	Group MC	4.43 ± 0.58	< 0.001
	Group F	10.93 ± 3.46	Group FC	4.35 ± 0.59	< 0.001
Insulin (µU/ml)	Group M	13.0 ± 5.06	Group MC	7.91 ± 1.91	< 0.001
	Group F	11.8 ± 5.68	Group FC	7.24 ± 1.84	< 0.001
HOMA-IR	Group M	5.02 ± 1.31	Group MC	1.06 ± 0.24	< 0.001
	Group F	4.78 ± 1.63	Group FC	1.03 ± 0.27	< 0.001

Lipid Profiles Measurements:

The result show statistically significantly increase ($p<0.001$) in TG, LDL-c and VLDL-c, while the HDL-c was found to be significantly decrease ($p<0.001$) ,(table 4) and no significant increase or decrease ($P > 0.05$) in lipid profiles in compassion between group M and group F.

Table (4): Mean total cholesterol, HDL-c, TGs, VLDL-c and LDL-c Concentrations in diabetic patients(type 2) and control groups.

Parameter	Patients			Controls		P -value
		Mean ± SD	P-value		Mean ± SD	
TC (mmol/L)	Group M	5.28 ± 0.142	0.154	Group MC	4.32 ± 0.51	< 0.001
	Group F	5.06 ± 0.111		Group FC	4.28 ± 0.48	< 0.001
TG (mmol/L)	Group M	2.7 ± 1.06	0.173	Group MC	1.42 ± 0.31	< 0.001
	Group F	2.49 ± 0.73		Group FC	1.43 ± 0.26	< 0.001
LDL (mmol/L)	Group M	3.2 ± 0.75	0.427	Group MC	2.57 ± 0.58	< 0.001
	Group F	3.1 ± 0.69		Group FC	2.39 ± 0.56	< 0.001
VLDL (mmol/L)	Group M	1.24 ± 0.52	0.136	Group MC	0.643 ± 0.142	< 0.001
	Group F	1.13 ± 0.33		Group FC	0.67 ± 0.138	< 0.001
HDL (mmol/L)	Group M	0.89 ± 0.18	0.122	Group MC	1.15 ± 0.25	< 0.001
	Group F	0.97 ± 0.24		Group FC	1.17 ± 0.2	< 0.001

Non High density lipoprotein cholesterol measurement:

The results exhibit significant increase (P <0.001) in Non HDL-c between patient groups and control groups and no significant (>0.05) difference between patient groups as in the table (5).

Table (5): Non HDL-C concentration in patient groups and control groups.

Parameter	Patients			Controls		
	Groups	Mean ±SD	P - value	Groups	Mean ±S D	P-value
Non –HDL c (mg/L)	Group M	4.42 ± 0.86	0.323	Group MC	3.07 ± 0.51	< 0.001
	Group F	4.17± 0.82		Group FC	2.91 ± 0.55	< 0.001

Retinol binding protein4:

The results exhibit significant increase (P <0.001) in RBP4 between patient groups and control groups (table 6), and no significant (>0.05) difference between patient groups.

Table (6): Mean + SD of RBP4 concentration in patients groups and control groups.

Parameter	Patients			Controls		
	Groups	Mean ±SD	P -value		Mean ±S D	P -value
RBP4 (mg/L)	Group M	76.4 ± 9.4	0.234	Group MC	54.7 ± 8.7	< 0.001
	Group F	73.8 ± 10.2		Group FC	53.2 ± 9.6	< 0.001

Relationship of retinol binding protein4 with insulin resistance:

The correlation of RBP4 with serum insulin and HOMA-IR were determined by correlation coefficient (r), in group M which show significant positive correlations of RBP4 concentration with fasting insulin concentration and HOMA-IR as exhibited in the table (7).

Table (7) Correlation between RBP4 with insulin level and HOMA-IR in diabetic patient groups

Parameter	Patients groups	Insulin level		HOMA – IR	
		r	P- value	r	P- value
RBP4 (mg/L)	Group M	0.514	0.0012	0.536	0.0034
	Group F	0.502	0.001	0.474	0.02

Relationship between RBP4 and Total Cholesterol, Triglycerides, LDL-c and HDL-c

The results exhibits a significant positive correlations between RBP4 concentration and TC, TG and LDL-c concentrations respectively, and negatively with HDL-c in group M and F, such as in table (8) .

Table (8): Correlation between RBP4 with TC, TG, LDL-c and HDL-c in diabetic patient groups

Parameters / Patients groups		RBP4 level	
		r	P. value
TC	Group M	0.486	0.001
	Group F	0.443	0.004
T.G	Group M	0.388	0.013
	Group F	0.467	0.002
LDL-c	Group M	0.385	0.014
	Group F	0.435	0.005
HDL-c	Group M	-0.424	0.006
	Group F	-0.453	0.003

Glucose Transporter-1 Genotyping :

TheXbaI polymorphism of GluT1 gene was determined by polymerase chain reaction (PCR). The result of amplification by PCR process which was conducted to define the G/T polymorphic site by using specific primer resulted in one band when visualized on agarose – gel electrophoresis. These band was 1.1 kilo base pair (kbp) representing presence of G/T polymorphic site in the GluT1 gene, as in figure (2).

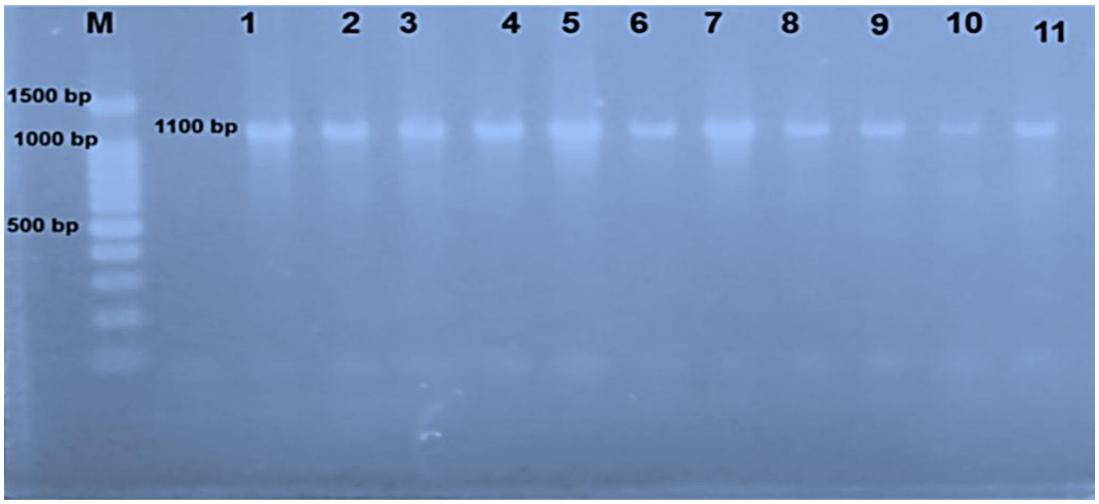

Fig. (2): Electrophoretic pattern of the GLUT-1 genotyping represents the XbaI genepolymorphism, Lane M: DNA ladder (marker), Lanes (1-11) are representXbaI polymorphism. Amplification product appeared as band of about 1100 bp.

After PCR product was digested with XbaI enzyme, the 1100-bp PCR fragment divided into 900 bp and 200 fragments. The allele was designated either G or T depending on whether the XbaI restriction site was present or absent, respectively, as in figure (3).

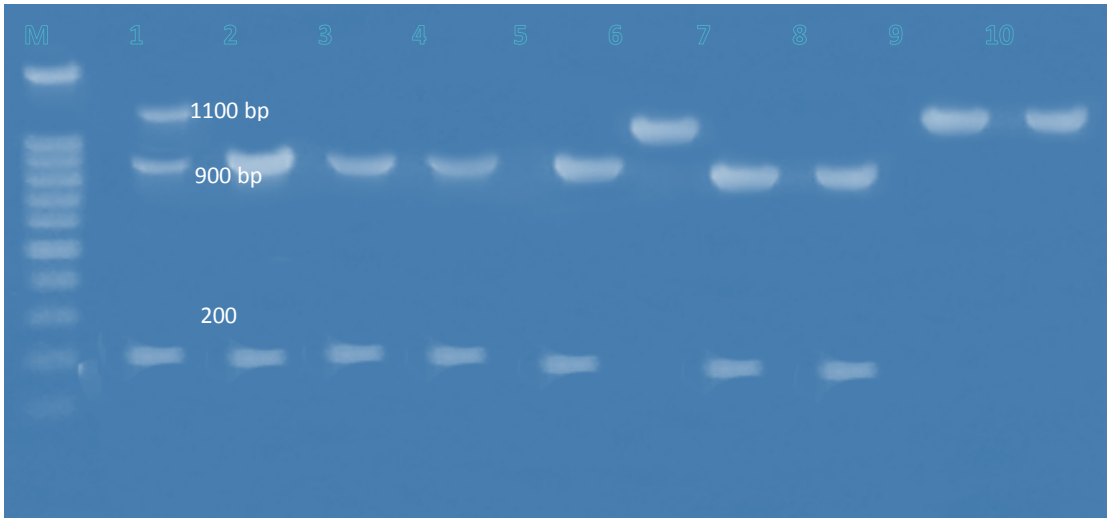

Fig.(3): Pattern of RFLP for glucose transporter-1 gene polymorphism. Lane M: DNA Ladder Lane 1 XbaI (-/+) genotype, lane 2, 3, 4, 5, 7, 8: XbaI

Genotype and Allele Frequency of Glucose Transporter-1 Gene :

Table (9) shows the genotype and allele frequencies of XbaI gene variant.

Table (9): Genotyping of GLUT-1 gene polymorphism with allele frequency.

Groups		Genotype			Allele frquency	
	No.	XbaI (-/-)	XbaI (-/+)	XbaI (+/+)	XbaI(-)	XbaI(+)
Group M	40	12 (30%)	7 (17.5%)	21 (52.5%)	39%	61%
Group F	40	14 (35%)	10 (25%)	16 (40%)	47.5%	52.5%
Group MC	40	3 (7.5%)	10 (25%)	27 (67.5%)	20%	80%
Group FC	40	2(5%)	8 (20%)	30 (75%)	15%	85%

In order to evaluate the significance of these results, the genotypic distribution in both the control groups and diabetic groups, table 10.

Table (10) Glucose transporter -1 gene polymorphism in patient andcontrol groups.

Genotype	Group M	Group MC	Odds ratio	CI (95%)	P – value
XbaI (-/-)	12	3	5.14	1.36 - 19.4	0.015
XbaI (+/-)	7	10	1.83	0.64 -5.2	0.25
XbaI (+/+)	21	27	1 reference		
	Group F	Controls FC			
XbaI (-/-)	14	2	13.12	2.64 - 65.07	0.0016
XbaI (+/-)	10	8	2.34	0.772 - 7.11	0.13
XbaI (+/+)	16	30	1 reference		

Endothelial Nitric Oxide Synthase Genotyping:

The resultsshows band in 248bp,these indicated to the presence of G894T polymorphic site in eNOS gene, figure 4.

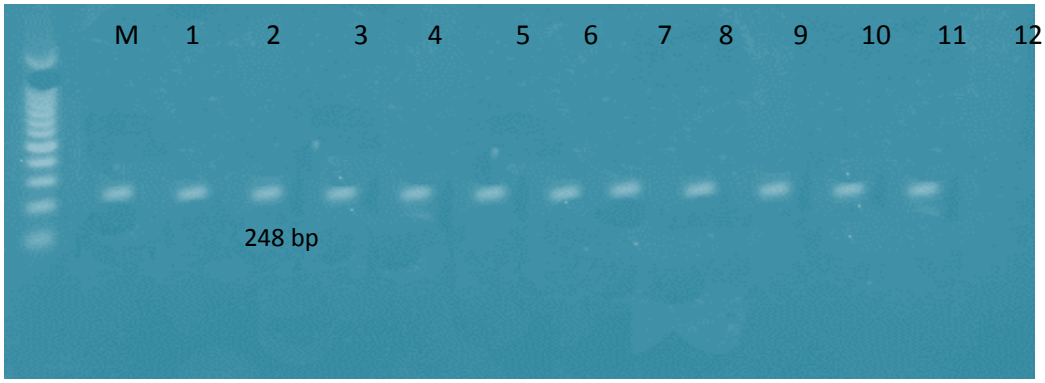

Fig.(4): The eNOS G894T genotyping (Electrophoretic pattern) represents the G894T gene polymorphism, Lane M: DNA ladder, Lanes (1-12) G894T polymorphism, (Amplification product band 248 bp).

The MobI enzyme was used to digested the product after PCR, and thisseparated into two fragment (158 bp and 90bp). In this case the allele was designated either T or G ,(figure 5).

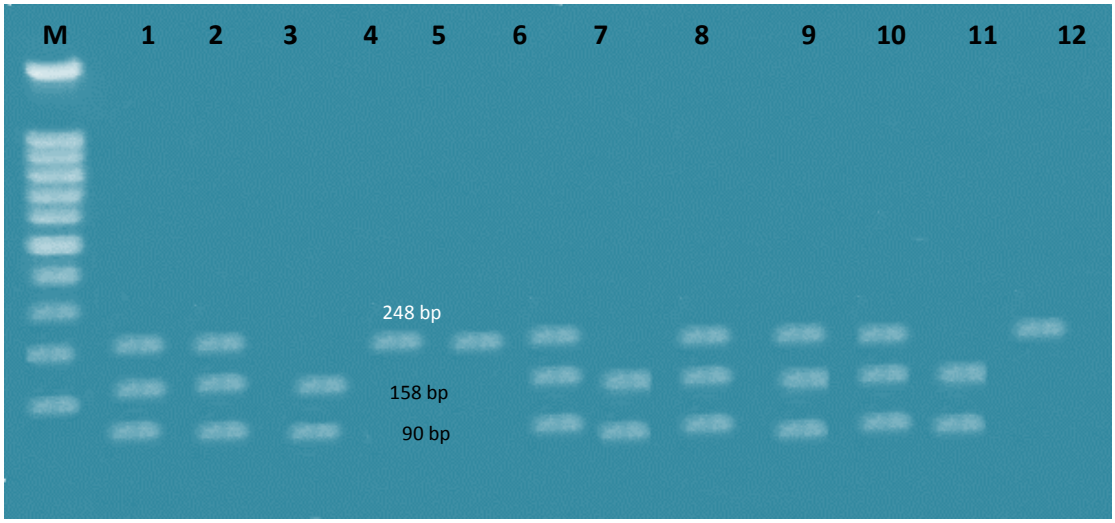

Fig. (5): A RFLP pattern of endothelial nitric oxide synthase gene polymorphism. Lane M: DNA Ladder (marker), Lane 1, 2, 6, 8, 9, 10 GT(+/-) genotype, lane 4, 5, 12 GG(-/-) genotype; lane 3, 7, 11 TT (+/+) genotype.

The genotype and allele frequencies of the G894T gene variant, table 11

Table (11): Genotyping of eNOS gene polymorphism with allele frequency.

Groups		Genotype			Allele frequency	
	No.	TT	G/T	GG	T	G
Group M	40	7 (17.5%)	21 (52.5%)	12 (30%)	43.75%	56.25%
Group F	40	9 (22.5%)	20 (50%)	11 (27.5%)	47.5%	52.5%
Group MC	40	8 (20%)	6 (15%)	26 (65%)	17.5%	82.5%
Group FC	40	10 (25%)	7 (17.5%)	23 (57.5%)	33.75%	66.25%

In order to evaluate the significance of these results, the genotypic distribution in both patient groups and control groups, table (12).

Table (12): eNOS gene polymorphism in patient andcontrol groups.

Genotype	Group M	Group MC	Odds ratio	CI (95%)	P – value
TT	7	8	1.89	0.55 - 6.44	0.305
GT	21	6	7.58	2.43 - 23.62	<0.001
GG	12	26	1 reference		
	Group F	Group FC			
TT	9	10	1.88	0.59 - 5.95	0.28
GT	20	7	7.27	2.40 - 22.02	<0.001
GG	11	23	1 reference		

Discussion

BMI represents a relative measure of body adiposity and it is a well - known fact that diabetics lose weight as the disease develops due to insulin deficiency and or IR which causes increased lipolysis and proteolysis by the effects of catecholamines and other counteract hormones[19].The results showed elevated level of fasting blood glucose in patient groups compared with control groups is due to that blood glucose is not utilized by all tissues leading to hyperglycemia which agree with Suilbert R[20], T2DM is preceded by a long period of asymptomatic extravagant blood glucose that may additionally lasting for years.

In this studythere is a relationship between fasting glucose and HbA1c level in patients and this agree with Ndiaye A.*et al* study, that the blood glucose and HbA1c concentrations frequently increase in patients[21].Through HbA1c can be monitor long-dated glycemic control in diabetic patients, and reflect the insulin affectability over the former weeks or months[22].According to these results elevated insulin level in diabetic groups as result of impaired response of tissues that dependent on insulin to uptake glucose from blood and extracellular fluid this known as insulin resistance which agree with Christian Weyer[23]. Many potential studies have shown that in insulin resistance, the cells are unable to respond to motivation by way of insulin efficiently, precedes un onset of T2DM by many years [24].The insulin signaling deficiency are linked to GLUT4 synthesis[25]. The results exhibited the dyslipidemia in diabetic subjects in contrast with controls this as result of impaired of insulin action on activity of lipoprotein lipase and hepatic overproduction of massiveVLDL particles which agree with Markku Laakso[26]study and S. Dahal*et al* study[27]. T2DM is correlated with a group of interconnected plasma lipid and lipoprotein irregularities, involving decreased HDL-c, a predominance of small dense LDL and elevated triglycerides[28].

The increase in VLDL-c occurred in patients due to increasing the glucose for VLDL-cholesterol produced and shacklein lipoprotein lipase action to clearance of VLDL-c from the peripheral circulation [29]. The raising of large VLDL particles begins a series of occurrences leading to the formation of small dense LDL and HDL has focused the assembly of VLDL particles on the spotlight as a possible offender of diabetic dyslipidemia[30]. High plasma TG level is most frequent lipid abnormality in IR and T2DM[31].

Our results exhibited that high levels of Non HDL-c in diabetic patients in both groups compared with control group, elevated Non HDL-c cocntrations in diabetic patients and this elivation may cause dyslipidemia ,micro angiopathy, micro- and macrovasecular diseases and risk factors for diabetic nephropathy.Marcovecchio *et al*.[32]showed there is association between microalbuminuria and high level of non HDL-c and suggested that non HDL-c may be a predictive marker for diabetic nephropathy.Non HDL-c manifest a higher predictor of vascular outcomes[33]. Non HDL-c levels associated with the episode of atherosclerosis in T2DM and it can be a simple and cumulative marker of cardiovascular disorder and Non HDL-c could be deemed as a sign for atherogenicity[34].

According to the result increasing of RBP4 concentration in diabetic patients because it increases in individuals with IR and T2DM as a result of its' role in causing IR and then progress to diabetes mellitus, this agree with Mostafaie N. study[35] that RBP4 plays a role in biological mechanisms that are responsible for IR and

development of T2DM, and disagree with Von Eynatten M. *et al* study[36]that RBP4 does not seem to be a valuable marker for identification of the metabolic syndrome or IR in male patients with T2DM.Studies have presented that plasma RBP4 levels are greater in casualties with DM than those without DM[37]. Entirely inconsistent results in human studies, RBP-4 concentrations increased in obese individuals, and higher levels correlated with lower insulin sensitivity and another components of the metabolic syndrome[38].

Determination of the function of retinol in the effects of RBP4 on IR is crucial for discerning the mechanisms of RBP4 action. Independently, RBP4 can act of retinol to weaken signaling of insulin in adipocytes indirectly, by inducing pro-inflammatory cytokine production from macrophages[39].The results show association between RBP4 with high cholesterol, triglycerides, LDL-c concentration and low HDL-c concentration due to the actuality it effectiveness on lipid metabolism specially growing VLDL-c production and in diabetic causalities activity of lipoprotein lipase is decreased due to diminished insulin or decreased response to insulin activity this agree with Broch M. *et al*,[40]that RBP4 associated with lipid parameters.

The effect of RBP4 on lipid concentrations, especially triglycerides, may be intervened through its effectiveness on metabolism of hepatic fatty acids, which regulates displaying of genes concerned in metabolism of lipid [41].Further research have counseled that RBP4 may play a more imperative role in lipid metabolism, as proved by many relations between RBP4 levels and serum lipids, and autonomous correlations of RBP4 with proatherogenic lipoproteins and key enzymes of lipoprotein metabolism in sufferers with and without metabolic syndrome indicate that RBP4 maybe play a role in lipid metabolism[42].

Our results exhibit about 30% of diabetic male patients and 35% of diabetic female patients have XbaI (-/-) polymorphism, and our hypothesis it is related to DN, they may have DN in future according to many studies like Gutierrez *et al* evaluated the XbaI polymorphism in the susceptibility to micro vascular complications in T2DM[43]. The genetic study in Chinese patients with T2DM, the XbaI (-) allele bestowed a significantly higher susceptibility to diabetic kidney disease. In the case control study, the oftenness of the XbaI genotype was compared between patients with DN (urinary albumin excretion > 200 μg/min) and without. The oftenness of the XbaI (-) allele carriers in patients with nephropathy was higher than in patients without renal complication[44].

The XbaI polymorphism is in linkage imbalance with other position which does have functional implications at the protein level and can function an etio-pathogenic role in disease[45]. From the results, the difference in structure of the basis around XbaI, may describe the various results in different populations.

Our results presented that heterozygote G/T allele in patients more frequently in tow patient groups in comparison with control groups and prospect that the GT increases the peril of DN for T2DM because abnormal synthesis of NO due to defect in eNOS , which this agree with Ahluwalia TS *et al*., [46] that eNOS (GT) genotype was found to be related to the rapid decline of renal function and a risk factor for end stage renal disease and Zintzaras *et al*[47], in a meta-analysis, showed that G894T NOs gene polymorphism is correlated with DN in East Asians patients. Shokri A. *et al*. suggested that eNOS polymorphisms can depict genetic determining factor for developing DN in type 2 diabetic patients[48].

The eNOS gene is expressed by vascular endothelial cells and variation in the NOS3 gene encoding eNOS alters nitric oxide production. Endothelial malfunction contributes to the progression of DN due to a decrease in production of active nitric oxide[49].In humans, up-regulated eNOS expression in glomerular endothelium was demonstrated in nephropathy patients with T2DM[50]. Experimental and clinical evidences propose that renal eNOS expression and activity are increased early after onset of diabetes, possibly mediating vasodilatation and hyper-filtration; however, they are decreased with prolonged diabetes and the resulting vascular NO deficiency may facilitate the evolution of DN[51,52].

The most clinically pertinent polymorphisms were depicted in the eNOS gene is the 894G > T exchange in exon 7 [53], this polymorphism associated DN, ranging from an increase of albuminuria up to end-stage renal disease in diabetic patients on hemodialysis[54-56]. DN in North ,Asian, Indian patients with T2DM. El-Din reported that TT genotype of eNOS had correlation with increased peril of end stage renal disease in patients with T2DM, so it maybe a practical marker for recognition of high peril patients with DM[57,58].

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