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LETTERS

Impact of resistin gene polymorphism on insulin resistance and Type 2 diabetes in Iraqi Babylon province patients

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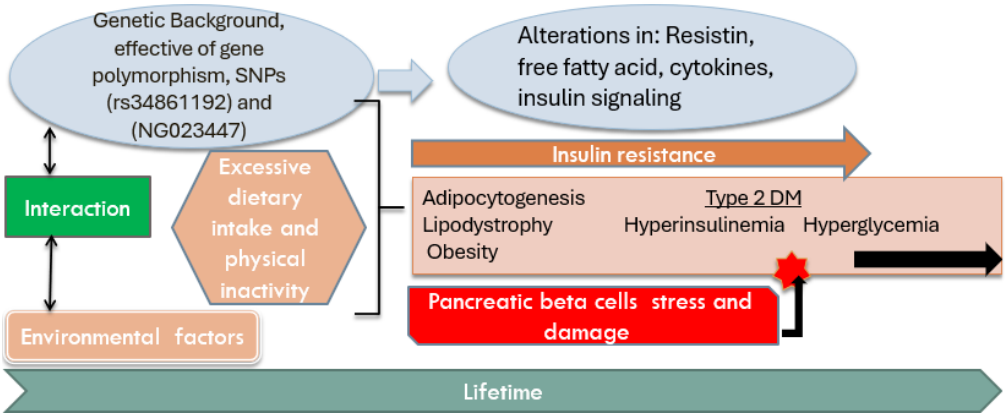
Article

ABSTRACT



Resistin is cysteine-rich polypeptide produced by adipocytes and macrophages. This study aims to assess the role of resistin and its gene polymorphisms (rs-34861192 G>A, NG-023447 C>G) as potential link between obesity and insulin resistance in the development of T2DM. Blood samples were collected from 120 participants (60 control are divided into 30 normal weight and 30 obese without T2DM) and (60 patients of Type 2 dm DM) are divided into 30 normal weight and 30 obese). Resistin and insulin levels were increased significantly in the patients' group ($p<0.05$). Gene analysis indicated that rs-34861192 was associated significantly ($P<0.01$) with T2DM in dominant, recessive, and co-dominant models. The rs-34861192 AA genotype showed a significant difference in normal-weight and obese T2DM compared to control ($P<0.001$) only. The significant difference of GG genotype in normal-weight patients than control exclusively. In the diabetic patients, mutant genotype (AA) of rs34861192 was associated with circulating resistin level. The expression of retn gene was high. Genotype AA of rs- 34861192 was correlated positively with folding change. Mutant AA of rs-34861192 G>A plays an important role in development of T2DM through its effect on resistin levels in the circulation that considered as a major factor for developing T2DM.

Keywords: Insulin, Resistin, Obesity, FBS, HbA1c, IR, retn gene, SNPs, gene expression

INTRODUCTION

Type 2 dm diabetes mellitus (T2DM) is recognized by defective insulin secretion, unresponsiveness of the target tissues to insulin, and insufficient production of insulin by β cells of pancreas, resulting in high blood glucose levels. T2DM constitutes about 90% of all diabetic patients [1,2]

According to data based on median age and sex differences, the prevalence of T2DM is higher in men than women. T2DM is developed later in life, due to systemic dysfunctions in metabolic homeostasis. In addition to genetic causes that play a substantial role in predisposing persons to T2DM, whereas unhealthy eating behaviors and sedentary life style work as strong triggers.[3,4] T2DM is heterogeneous and very complex disease due to involve numerous pathophysiological mechanisms that affect both the pancreas and metabolic organs, leading to challenging for effective treatment. Insulin resistance is recognized by impairing biologic response to insulin stimulation of target tissues, leading to increase insulin production and hyperglycemia.[5] IR is predominantly an acquired condition connected to excess body fat and genetic causes.[6] The important role of resistin which is a small secretory molecule has been implicated the progression of insulin resistance under obese condition.[7]

Resistin is a 12 k Dalton cysteine-rich polypeptide produced by adipocytes and macrophages. It consists of 108 amino acid and circulates as a dimeric protein consisting of two 92-amino acid polypeptides.[7,8] Resistin gene is located on chromosome 19 and its protein participates in insulin sensitivity and function.[9] Monocytes and macrophages are the main site for producing resistin and it is directly produced by adipocyte in small amounts. So that the molecular pathways involved in inflammatory,

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metabolic, autoimmune, and cardiovascular diseases is modified by resistin.[10] It prohibits insulin ability to stimulate cellular glucose uptake by muscle and adipose tissues and plays an important role in obesity, insulin resistance, diabetes.[11] The secretion of resistin is increased with obesity, and it inhibits insulin-induced glucose uptake by acting directly on adipocytes.[12] The effect of resistin as pro-inflammatory factor may be mediated by binding to TLR4. This binding causes activation of NF-κB in macrophages through the JNK and p38 MAPK pathways; these participate to the development of insulin resistance and inflammation, closely associated with obesity and related to metabolic diseases.[13,14] This study was designed to assess the role of resistin as potential link between obesity and insulin resistance in the pathogenesis of T2DM. Most cases of T2DM are closely related to genetic and environmental risk factors,[11,12] and their interactions.[13] Previous genome-wide association studies[15-18] have identified numerous genetic polymorphisms and rare genetic variants associated with slight or significant effects on T2DM, suggesting that the disease results from complex interactions between genetic mechanisms and environmental factors.

MATERIALS AND METHODS

Study data

The present design is a case control study. Blood sample was collected after an overnight fasting from 120 participants during the period (1/3/2022 to 20/9/2022). Samples of T2DM patients (30 obese and 30 normal weight) were obtained from Marjan medical city and teaching hospital /Hilla city that diagnosed by specialist endocrinologist while remaining samples were 60 subjects (30 obese without T2DM and 30 apparently healthy control). Practical side was performed at the laboratory biochemistry department/College of medicine/University of Babylon. Three milliliters of blood were placed in the gel tube. Blood was centrifuged for 15 minutes at 3000 xg. Serum was collected then stored at -20 C° until analyses for the determination of insulin, resistin, IR, HbA1c, glucose concentrations. The samples were putted in a box containing ice for frozen samples after collected and transferred it to the laboratory. Insulin levels were measured by human insulin (INS) Biont ELIAS KIT and resistin levels were measured by human resistin BIONT ELIAS KIT, while FBS was measured by using the Roche COBAS c311 by used glucose COBAS kit and HbA_1c by using COBAS INTEGRA© 400 plus by used COBAS HbA1c kit. The two SNPS (rs 34861192 and NG 023447) and the expression of *retn* gene was studied by using HRM and ARMs PCR. The concentration of DNA and RNA were measured by nano drop 2000.

Ethical Statement

Each volunteer enrolled to this study has given written informed permission. This research received ethical approval (DSM/HO-65031) for scientific research from the Ministry of Health MOH and Ministry of Higher Education and Scientific Research MOHESR ethics committees in Iraq.

Biochemical analysis

Insulin and resistin were measured by enzyme linked immunosorbant assay (ELISA) kit, FBS and HbA_1c were measured by spectrophotometer. IR were calculated using the following equation:[19]

HOMA-IR = fasting insulin (μIU/ ml) * fasting glucose (mg/dl) / 405

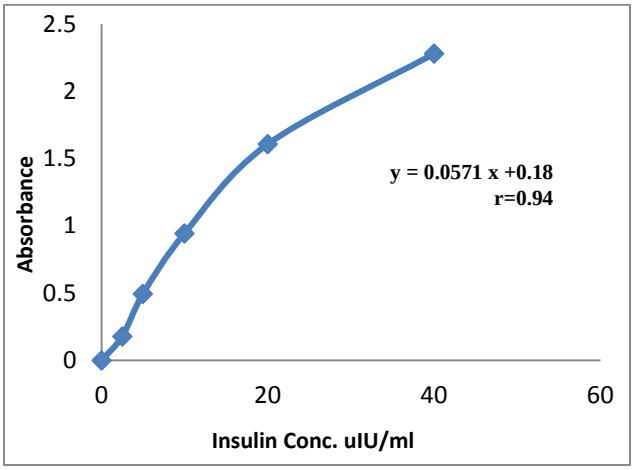

Figure 1 : Standard curve for insulin by ELISA.

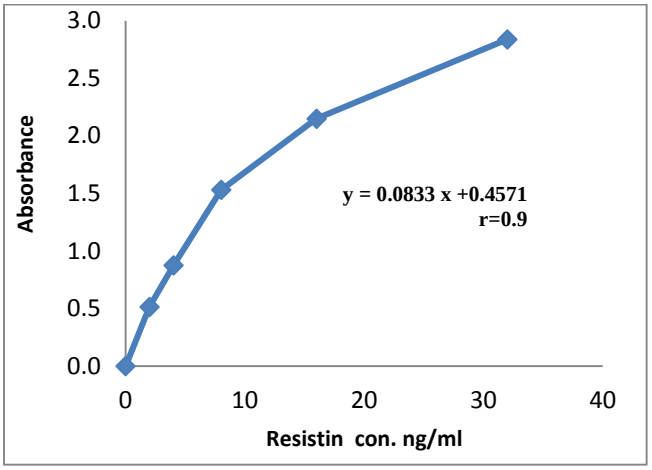

Figure 2. Standard curve for resistin by ELISA.

Statistical analysis

Statistical analysis was performed using SPSS IBM, version 26.0. The normality of data distribution was tested by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Present data were Non-normally distributed. Mann-Whitney U test was applied for comparison between two groups that expressed as median, while test statistic (mean rank difference) of Krusskal Wallis Post Hock test was used for multi-comparison among studied groups. Correlations between variables were performed using Spearman correlation. ROC analysis was used to determine the diagnostic markers for studied variables. The area under the curve (AUC) provides a useful tool to compare different biomarkers good and excellent range from (80-100). ($P \leq 0.05$) consider as a significant value. Test for Hardy-Weinberg equilibrium of allelic or

genotypic association in cases versus control were evaluated by Chi – square (x^2) test, this analysis was performed for all genotypes in this study using Hardy-Weinberg equilibrium online calculator.

To assess the predictability of T2DM, logistic analysis of both SNPs was applied, this yielded odds ratio (OR). Also, the 95% confidence interval was calculated which is good estimator for the significance of the OR; when the value of "one" included within interval, this is an indicator that the OR is not significant.

RESULTS

Results of (Table 1) shows standard deviation, median, minimum, maximum and P value of age among control and patient groups, there was no significant (P >0.05) among controls and patients groups.

Table 1. Comparison of age between patients and control using Mann-Whitney U test.

parameters	Studied groups	median	minimum	maximum.	P values
Age (years)	Control Patients	49 50	39 40	65 65	0.127

Present analysis in (Table 2) shows the test statistic, standard error and standard deviation of BMI among the studied subgroups. There were no significant (P>0.05) changes in BMI between (non-obese without T2DM and non-obese T2DM) and (obese without T2DM and obese T2DM). While other comparison among studied groups were recorded significant (P< 0.05) variation.

Table 2. Krusskal Wallis Post Hock multi-Comparison for body mass index among studied groups.

Para meters	groups	Study groups	Test Statistic (mean rank difference)	SE	Std.	P values
BMI (Kg/m^2)	Non obese without T2DM	Non obese T2DM	-10.133	8.979	-1.129	.259
		Obese without T2DM	-60.133	8.979	-6.697	.000
		Obese with T2DM	-70.000	8.979	-7.796	.000
	Non obese T2DM	Obese without T2DM	50.000	8.979	5.568	.000
		Obese with T2DM	-59.867	8.979	-6.667	.000
	Obese without T2DM	Obese T2DM	-9.867	8.979	-1.099	.272

P value≤0.05 was significant, T2DM (type 2 diabetes mellitus), SE (Standard error) Std. (standard deviation), BMI (body mass index).

Data in table 3 shows the test statistic, standard error and standard deviation of hormonal parameters and IR among the studied subgroups. Present results were indicated significant differences between patient with T2DM and subjects (non-obese and obese) without T2DM., insulin, resistin, IR were significantly (P<0.05) elevated in T2DM patient compared to non-obese and obese without T2DM groups. But there was non-significant (P>0.05) differences in resistin between obese without T2DM and obese T2DM.

Table 3. Krusskal Wallis Post Hock multi-Comparison of hormonal parameters and IR among the studied subgroups

Parameters	GROUPS	Study groups	Test Statistic (mean rank difference)	SE	Std.	P values
Insulin (mIU/L)	Non obese without T2DM	Non obese T2DM	-19.600	8.971	-2.18	.029
		Obese without T2DM	-49.783	8.971	-5.54	.000
		Obese with T2DM	-74.683	8.971	-8.32	.000
	Non obese T2DM	Obese without T2DM	30.183	8.971	3.364	.001
		Obese with T2DM	-55.083	8.971	-6.14	.000
	Obese without T2DM	Obese with T2DM	-24.900	8.971	-2.77	.006
Resistin (ng/ml)	Non obese without T2DM	Non obese T2DM	-37.117	8.981	-4.13	.000
		Obese without T2DM	-69.167	8.981	-7.70	.000
		Obese with T2DM	-70.583	8.981	-7.85	.000
	Non obese T2DM	Obese without T2DM	32.050	8.981	3.569	.000
		Obese with T2DM	-33.467	8.981	-3.72	.000
	Obese without T2DM	Obese with T2DM	-1.417	8.981	-.158	.875
IR	Non obese without T2DM	Non obese T2DM	-33.600	8.981	-3.74	.000
		Obese without T2DM	-59.083	8.981	-6.57	.000
		Obese with T2DM	-86.517	8.981	-9.63	.000
	Non obese T2DM	Obese without T2DM	-25.483	8.981	-2.83	.005
		Obese with T2DM	-52.917	8.981	-5.89	.000
	Obese without T2DM	Obese with T2DM	-27.433	8.981	-3.05	.002

P value≤0.05 was significant, T2DM (type 2 diabetes mellitus), SE (Standard error) Std. (standard deviation), IR (insulin resistance).

Table 4 shows the test statistic, standard error and standard deviation of biochemical parameters among studied subgroups. Present results were indicated significant differences between patients with T2DM and subjects (non-obese and obese) without T2DM., FBS, HbA1c were significantly (P<0.05) elevated in T2DM patients compared to non-obese and obese without T2DM groups. But there was non-significant (P>0.05) change of both FBS and HbA1c between obese without T2DM and obese T2DM.

Table 4. Krusskal Wallis Post Hock multi-Comparison of biochemical parameters among the studied subgroups

Para meters	GROUPS	Study groups	Test Statistic (mean rank difference)	SE	Std.	P values
FBS (mg/dl)	Non obese without T2DM	Non obese T2DM	-22.333	8.973	-2.489	.013
		Obese without T2DM	-66.933	8.973	-7.459	.000
		Obese with T2DM	-75.400	8.973	-8.403	.000
	Non obese T2DM	Obese without T2DM	-44.600	8.973	-4.970	.000
		Obese with T2DM	-53.067	8.973	-5.914	.000
	Obese without T2DM	Obese with T2DM	-8.467	8.973	-.944	.345
HbA1c %	Non obese without T2DM	Non obese T2DM	-24.300	8.974	-2.708	.007
		Obese without T2DM	-68.200	8.974	-7.599	.000
		Obese with T2DM	-76.100	8.974	-8.480	.000
	Non obese T2DM	Obese without T2DM	-43.900	8.974	-4.892	.000
		Obese with T2DM	-51.800	8.974	-5.772	.000
	Obese without T2DM	Obese with T2DM	-7.900	8.974	-.880	.379

P value≤0.05 was significant,T2DM (type 2 diabetes mellitus),SE (Standard error) Std. (standard deviation).,FBS(fasting blood glucose),HbA1c(hemoglobin A1c).

Spearman Correlation was used to determine whether the relation among present parameters related with development of IR, present results were described in table (5).

Receiver Operating Characteristic Curve of resistin

The area under the curve (AUC) was 0.87, 95%CI= (0.78 - 0.96), p-value =0.000. The sensitivity and specificity of the test at the cutoff value of were 75.6% and 58.1%, respectively. As shown in table (6) and Figure (2).

Table 5: Spearman Correlation Between the Levels of Parameters in patients with T2DM.

Variable		Insulin	Resistin	FBS	HbA1c	IR	BMI
Insulin mIU/ml	r		.947	.530	.246	.983	.988
	Sig.	.	.000	.000	.058	.000	.000
	N		60	60	60	60	60
Resistin ng/l	r	.947		.8	.247	.983	.924
	Sig.	.000		.000	.057	.000	.000
	N	60		60	60	60	60
FBS mg/dl	r	.530	.678		.147	.633	.456
	Sig.	.000	.000		.263	.000	.000
	N	60	60		60	60	60
HbA1c %	r	.246	.247	.147		.168	.166
	Sig.	.058	.057	.263		.061	.06
	N	60	60	60		60	60
IR	r	.983	.983	.633	.168		.963
	Sig.	.000	.000	.000	.061		.000
	N	60	60	60	60		60
BMI Kg/m^2	r	.988	.924	.456	.166	.963	
	Sig.	.000	.000	.000	.06	.000	
	N	60	60	60	60	60	

Table 6. Receiver Operating Characteristic (ROC) Curve of resistin

Area under the curve (AUC)	specificity	sensitivity	SE	P-value	95% (Confidence interval)
0.87	90%	76%	0.048	0.000	0.78 – 0.96

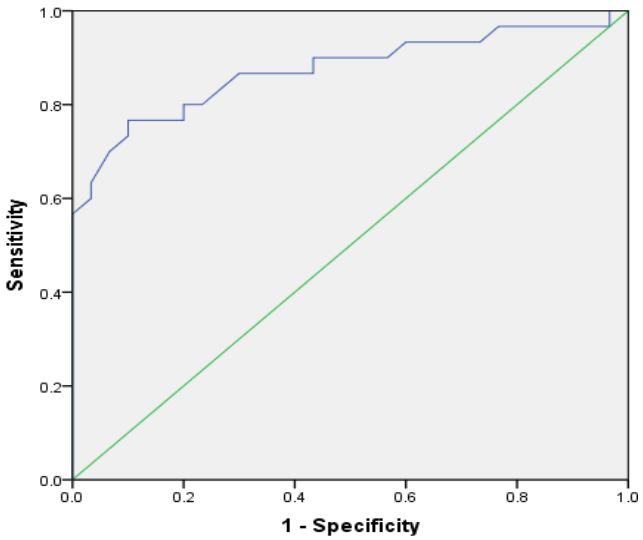

Figure 3. Roc Curve of resistin patient with T2DM and control.

Figure (4) shows high accuracy, of DNA bands of some studied samples that detected by agarose gel electrophoresis staining with low concentrations of a safe red fluorescent dye. A ratio of absorbance at 260/280 nm and 260/230 nm were used to measure the DNA and RNA purity that expressed as (mean ± SD) 1.82 ± 0.12 and 1.91± 0.08 for DNA, 1.88±0.2and 1.95± 0.14 for RNA respectively, the mean of DNA and RNA concentrations were 90.9 ± 2.9 and 79.3± 2.2 respectively. Data was demonstrated in Table(7).

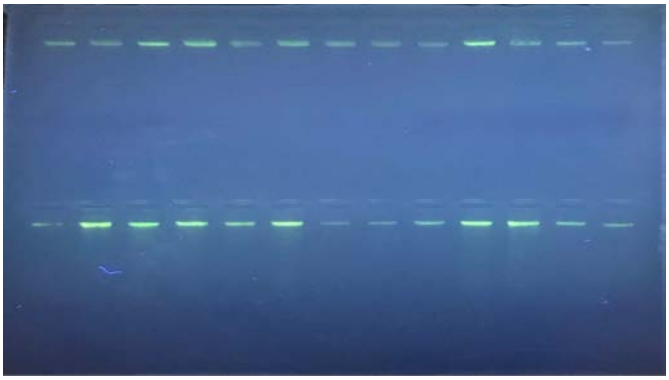

Figure 4: Detection the presence of genomic. (1-26) DNA extraction from frozen blood.

Table 7: Concentration and purity of deoxyribonucleic acid

DNA,RNA concentration and purity	No.	Mean ± SD
DNA concentration(ng/µl)	120	90.9± 2.9
DNA purity(260/280)	120	1.82±0.12
DNA purity(260/230)	120	1.91± 0.08
RNA concentration(ng/µl)	120	79.3± 2.2
RNA purity(260/280)	120	1.88±0.2
RNA purity(260/230)	120	1.95± 0.14

Figure (5) shows fluorescent signal of amplification curve for rs34861192 (G>A). Genotyping analysis by HRM was performed from 65˚C to 90˚C with a temperature increase of 0.2˚C/s.

Figure (5 and 6) show HRM assay melt curve results. The results were normalized to identify the genotype, the top, middle and bottom lines represent GG, GA and AA genotypes for control and patients respectively.

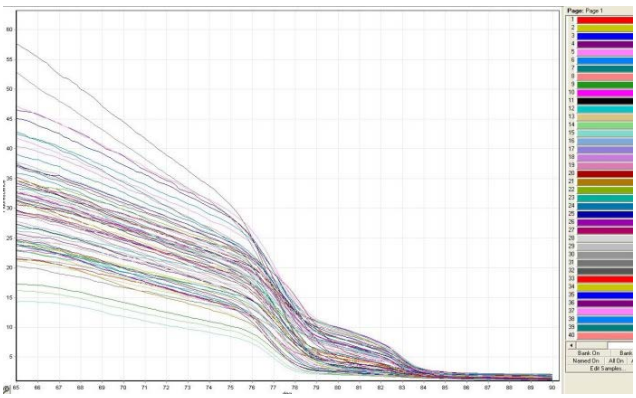

Figure 5: Amplification curve of rs34861192 G>A

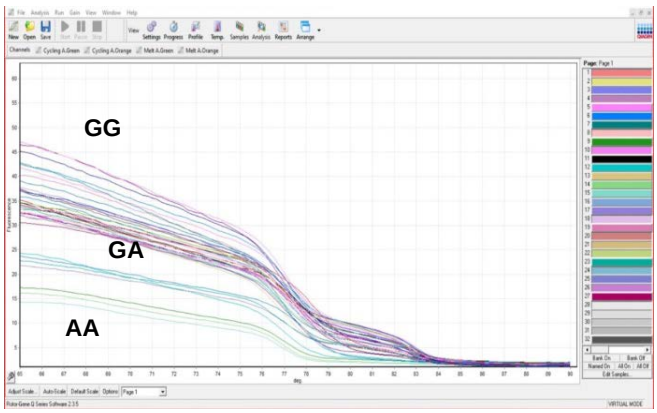

Figure 6: Genotype steps for control of rs34861192 G>A, GG wild, GA hetero, AA mutant for 1 run of PCR

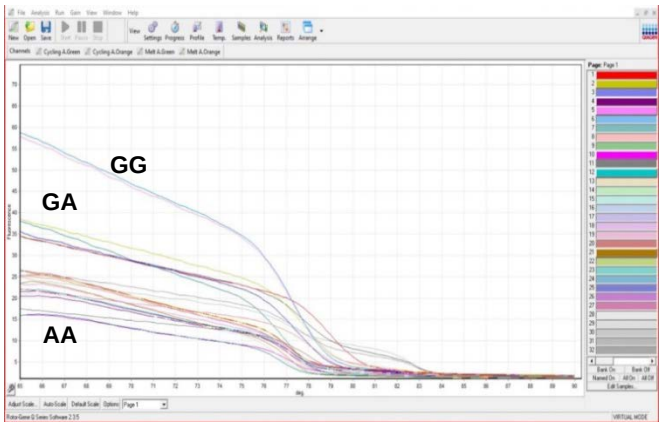

Figure 7: Genotype step for patients of rs34861192 G>A, GG wild, GA hetero, AA mutant for 1 run of PCR.

Figure 8 shows the amplification curve for NG023447 (C>G).

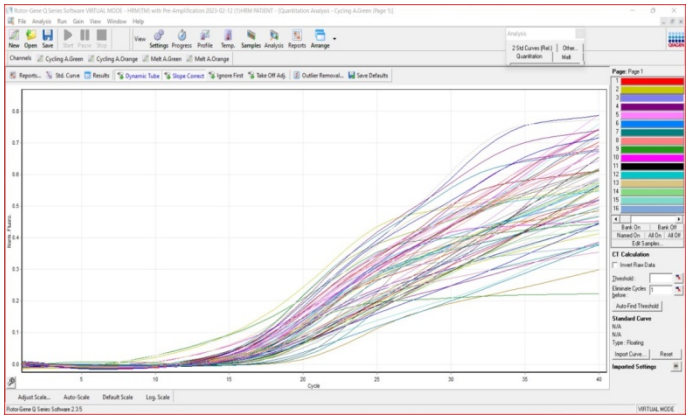

Figure 8: Amplification curve of NG023447 C>G.

ARM assay melting curve results were normalized to identify the genotype in figure (8) for control and patients of NG023447C>G.

Figure (9) and (10) show the amplification curve of *retn* gene expression -420 C>G in control and T2DM patients respectively.

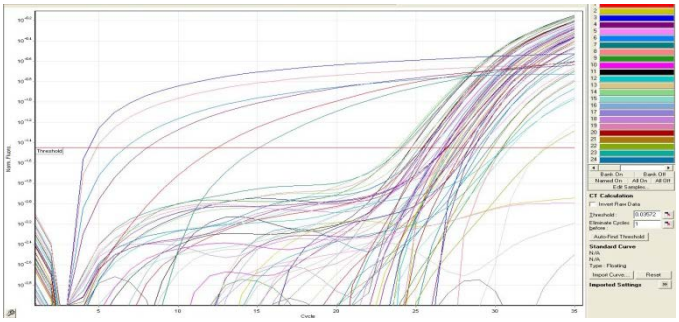

Figure 9: Amplification curve in control group.

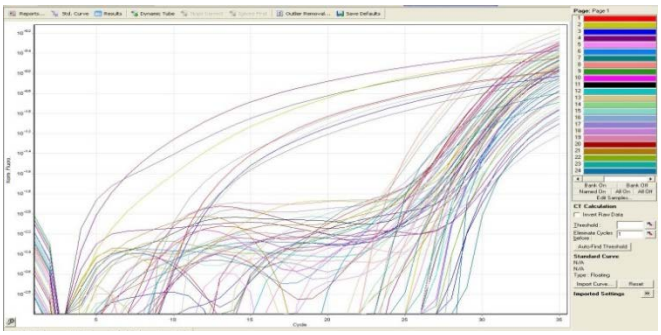

Figure 10: Amplification curve in T2DM group.

Table 8 shows allele frequency of retn gene variants rs34861192(G>A) and NG023447C>G in T2DM patients were represented [(20.8 and 24) %, P<0.0001, OR (95% CI) = 0.1(0.06 to 0.21)(0.05 to 0.183)]of G and C allele respectively and [(79 and 76)%, P<0.0001, OR (95% CI) = P<0.0001, OR (95% CI) = 8.2(4.5 to 14.7),9.8(5.4 to17.8)] of A and G allele respectively, while in control groups were represented (68.3 and 75)% P<0.0001, OR (95% CI) = 0.1(0.06 to 0.21)(0.05 to 0.183)]of G and C allele respectively and (31.6 and 25) % P<0.0001, OR (95% CI) = P<0.0001, OR (95% CI) = 8.2(4.5 to 14.7),9.8(5.4 to17.8)] of A and G allele respectively.

Table 8: Alleles frequency and allelic association of rs34861192 G>A and NG023447 C>G of *retn* gene polymorphism between Patient and control groups.

Retn SNPs	Allele	Frequencies (%)		Odd ratio (95% CI)	P value
		Control (n=60)	Patient (n=60)		
rs-34861192	G	68.3% (n=82)	20.8% (n=25)	0.1 (0.06 to 0.21)	< 0.0001
	A	31.6% (n=38)	79% (n=95)	8.2 (4.5 to14.7)	
NG-023447	C	75% (n=90)	24% (n=28)	0.1 (0.05 to 0.183)	< 0.0001
	G	25% (n=30)	76% (n=92)	9.8 (5.4 to17.8)	

G- major allele, A-minor allele for rs 34861192, CI: confidence interval, SNP (single nucleotide polymorphism), P value ≤0.05 was significant.

Genotype frequencies of rs34861192 of *retn* gene polymorphism were not agreement with Hardy Weinberg Equilibrium (P<0.05) in T2DM patient and control groups as in Table (9). Whereas genotype frequencies of NG023447 of *retn* gene polymorphism were in agreement with Hardy Weinberg Equilibrium (P>0.05) in T2DM patient and control groups as in Table (10).

Table 9: Hardy-Weinberg equilibrium law of Retn gene polymorphism rs34861192 observed and expected genotype frequency for control and patient

Groups	GG	GA	AA	P value
Control	63% (n=38)	10% (n=6)	26% (n=16)	<0.05
Patient	16% (n=10)	8% (n=5)	75% (n=45)	<0.05

G- major allele, A-minor allele, P value ≤0.05 was significant.

Table 10: Hardy-Weinberg equilibrium law of Retn gene polymorphism NG023447 observed and expected genotype frequency for control and patient

Groups	CC	CG	CC	P value
Control	52% (n=31)	46% (n=28)	2% (n=1)	0.058
Patient	5% (n=3)	36% (n=22)	59% (n=35)	0.56

All data were conducted to assess the effects of polymorphism (SNP rs34861192 of retn gene variants [GG (wild type), GA (heterozygous type), and AA (mutated type)] on T2DM development, the results were summarized in Table (11) that indicated a higher risk model was recorded in genotypes AA co-dominant model which was [P<0.05, OR (95% CI) = 10.68(4.34 to 26.29)], followed by AA recessive model which was [P<0.05; OR (95% CI) = 8.2(3.6 to 18.7)], also GA-AA dominant model which was [P<0.05; OR (95% CI) = 8.6(3.6 to 20.3)]. While there was non-significant(p>0.05) association found in GA over all models.

Table 11: Association of rs34861192 genotypes with T2DM under different models of inheritance.

Model	Genotype	Control (No.60)	Patient (No.60)	Odd ratio (95% CI)	P. Value
Co. dominant	GG	70% (n=38)	16% (n=10)	References (OR=1)	
	GA	8% (n=6)	8% (n=5)	3.1 (0.79 to 12.5)	0.1
	AA	21% (n=16)	75% (n=45)	10.68 (4.34 to 26.29)	<0.0001
dominant	GG	70% (n=38)	16% (n=10)	References (OR=1)	
	GA-AA	30% (n=22)	83% (n=50)	8.6 (3.6 to 20.3)	<0.0001
Recessive	GG-GA	78% (n=44)	25% (n=15)	References (OR=1)	
	AA	21% (n=16)	75% (n=45)	8.2 (3.6 to 18.7)	<0.0001
Over dominant	GG-AA	91% (n=54)	91% (n=55)	References (OR=1)	0.75
	GA	8% (n=6)	8% (n=5)	0.8 (0.23 to 2.8)	

The comparison was also conducted in obese and non-obese T2DM to determine the effect of the obesity under three basic genotype, Table (12) was showed the highly significant difference (P<0.001) of AA genotype in non-obese patients and obese compared to control, while non-significant differences recorded among studied groups underline GA genotype.

Table 12: Genotype frequency of rs34861192 G>A of *retn* gene in patient and control subgroups (normal weight and obese).

Genotypes	Groups	Control	Patient	P. Value	
GG	Non Obese	24	9	References	
	Obese	14	1		
GA	Non Obese	1	3	0.08**	0.08*
	Obese	5	2	0.19***	
AA	Non Obese	5	18	0.0004**	0.01*
	Obese	11	27	0.001***	
Total	All subject	60	60		

*** between obese (patients and control), **between non obese (patients and control),* between non obese and obese in patients only, P value ≤0.05 was significant.

On the other hand, NG-023447 C>G SNP that described in Table (13) was showed the highly significant difference (P<0.001) of GG genotype in non-obese patients and obese compared with control. Also, there was a highly significant difference (P<0.01) of CG genotype non obese patients and control subject.

Table 13: Distribution to genotype frequency of NG-023447 C>G of Retn gene polymorphism between Patient and control group within subgroup.

Genotypes	Groups	Control	Patient	P. Value	
CC	Non Obese	22	2	References	
	Obese	9	1		
CG	Non Obese	8	13	0.009**	0.8*
	Obese	20	9	0.2***	
GG	Non Obese	0	15	0.0004**	0.4*
	Obese	1	20	0.0004***	
Total	All subject	60	60		

*** between patient and control as obese, ** between non obese (patient and control), * between non obese and obese in patient only

Table (14) described the probable influence of promoter *retn* gene SNP rs34861192G>A in serum resistin level of T2DM patients. Higher concentration of resistin was observed in mutant genotype (AA) that was significantly (p<0.05) associated with resistin level when compared with (GG)genotype.

Table (15) described the probable influence of *retn* gene SNP NG023447C>G in circulating resistin concentration of T2DM patients. Present results didn't show any significant (p>0.05) association between resistin level with all genotypes model.

Table 14. Krusskal Wallis Post Hock multi-Comparison of resistin level according to rs-34861192 G>A genotypes in T2DM patients.

Parameters	Genotype	Mean rank difference	SE	Std.	P values
Resistin (ng/ml)	GA-GG	0.145	9.417	0.015	0.988
	GA-AA	-13.055	8.240	-1.584	0.113
	GG-AA	-12.909	5.885	-2.193	0.028

Table 15. Krusskal Wallis Post Hock multi-Comparison of resistin with genotypes of NG-023447 C>G among the studied subgroups.

Parameters	Genotype	Mean rank difference	SE	Std.	P values
Resistin (ng/ml)	GC-CC	0.417	6.981	-.188	0.775
	CG-GG	-5.055	4.240	-1.584	0.34
	CC-GG	-10.44	3.885	-3.0	0.16

Table (16) described the probable influence of promoter *retn* gene SNP rs34861192G>A on the folding change of *retn* gene expression (gene of interest (GOI)) in T2DM patients. The results were expressed as mean±SD. Higher folding change was recorded in patients (156.8±17.66**)** when compared with the control (19.69±6.56).

Table 16. Krusskal Wallis Post Hock multi-Comparison of folding change with genotypes among the studied subgroups.

	House Keeping Gene	Retn "GOI"	ΔCT	ΔΔCT	Folding change
Average mean±SD CT Of Control	23.06±7.84	25.19±6.10	2.128	10±2.32	19.69±6.56
Average mean±SD CT of Patients	17.35±10.04	17.61±9.22	0.257	1.87±3.98	156.80±17.66

Table 17 described the probable influence of promotor *retn* gene SNP rs34861192G>A on the folding change of *retn* gene expression. Higher level of folding change was observed in mutant genotype (AA) that was significantly (p<0.05) altered when compared with (GG)genotype the mean rank difference (-23.456).

Table 17. Krusskal Wallis Post Hock multi-Comparison of folding change with genotypes of rs-34861192 G>A between control and patient.

Parameters	Genotype	Mean rank difference	SE	Std.	P values
Folding change	GA-GG	-8.350	9.561	-.873	0.382
	GA-AA	-6.806	5.1	-1.32	0.41
	GG-AA	-23.456	8.228	-2.851	.0040

DISCUSSION

Data of current study were recorded statistically significant elevation of resistin levels in obese patient with T2DM in comparison with non-obese T2DM, obese without T2DM and healthy controls. This finding is in agreement with other studies linking resistin with T2DM and the degree of obesity [20,21]. But other researchers don't observe the same finding.[22,23] Serum resistin levels was positively correlated with IR in present study of diabetic individuals. Previous research were reported the same results between the resistin levels and IR in the same line with present finding.[20,24] Serum resistin was correlated with insulin, FBS in diabetic patients that is in agreement to the studies that recorded the same result (Gharibeh MY, etal and Al-Harithy RN,etal).[20,24] Otherwise, this association was not determined in other studies.[20,22] The correlations between resistin with insulin, IR and FBS are may be referred to the nature of resistin due to it is a cysteine-rich peptide hormone which is directly connect obesity with diabetes. Resistin is secreted by macrophages of adipose tissue. It binds to TLR and promotes downstream inflammatory pathways like NFkβ, LRR, and NLRP-3. These steps further induce TNF-α and IL-6, that stimulate serine-threonine kinase which causes downregulate of IRS and, insulin receptor leading to amplify of insulin resistance in adipose tissue.[25]

The difference of BMI among current studied groups was used to determine its effects on resistin and determine the effects of later in insulin resistance. Such positive correlation was currently documented between serum resistin levels and BMI in diabetic patients (r= 0.988, P=< 0.05). Previous research were recorded correlation between resistin level and BMI in the same line with present finding.[20,26] Actually, resistin plays a pivotal role in inflammation process because of its expression and secretion from human macrophages is promoted by various pro-inflammatory stimuli,[27] Δ-DCN is one of resistin receptor that was detectable mainly in WAT, lung and bone marrow, and it is increased by obesity to regulate WAT expansion.[28,29] So that, adipose tissue is expanded resulting in adipocyte hypertrophy and the release of adipokines. The latter causes increased cytokine production, leading to adipose tissue dysfunction and impairment of glucose tolerance.[30]

All data have been added potential fact that obesity is associated with an increased level of serum resistin and is directly related with resistance to insulin [24]. From these observations, resistin is the factor that resist insulin function that supported by the data obtained of present study for rising of FBS, fasting insulin and IR in T2DM. The idea of such observation is when insulin reaches a critical level, the resistin might cause initiation of resistance to insulin or vice versa. Anyway, many of studies have documented that adipokines, such as leptin, resistin and adiponectin, is changed in T2DM and these may be contributed to the development of resistance to insulin [31]. Furthermore, present data of ROC analysis confirmed that resistin could potentially be used as biomarker for obese T2DM patients and differentiated them from non-obese T2DM patients and obese patient without T2DM with high sensitivity and specificity of resistin in this group.

All data were conducted to assess the effects of polymorphism (SNP rs34861192 of retn gene variants [GG (wild type), GA (heterozygous type), and AA (mutated type)] on T2DM development, a higher risk model was recorded in genotype AA, while in NG023447 the higher risk was recorded in GG genotype which. The *retn* gene polymorphisms results

Asano, H. *et al* (2010),[32] showed that significant association between rs34861192 polymorphism with T2DM the G allele was protective, and the frequency of allele A was significantly higher in T2DM patients compared to controls.

This study tries to revealed whether NG023447 in retn gene played a role in present case-control analysis. Allele frequency results indicates that C allele cannot be considered a risk factor in patients with (0R 0.1 CI 95% 0.05-0.183) and that allele G cannot be considered a protective factor in healthy subjects with (OR9.8 CI 95% 4.5-17.8).

Alleles frequency and allelic association of NG023447 C>G in retn gene revealed that there is highly significant difference between (Control and Patients) as shown in table (8), where the P-value was less than 0.0001. From the results obtained, it can be said that the mutation that revealed in study has heterogeneous effects on the community and for all groups (patients and healthy people).

The current study results are similar to the case control study of Asano, H. et al (2010)[31] that reported higher frequency of the mutant AA genotypes of rs34861192 SNP, and exhibited a significant association with the risk of T2DM mainly in obese patients. Present result documented that mutant AA highly frequent also in obese patients.[31]

Another findings of present work demonstrated that higher circulating levels of resistin was observed in AA genotype, these similar to(Wei Liu, etal and Asano H. etal) [31,32].These authors explained many genetic factors such as (DNA methylation) contribute to the circulating changes in resistin, particularly the SNPs in the promoter region of the resistin gene such as rs34861192, rs3745368 and rs1862513.

One of the strong point of present research is the measurement of *retn* gene expression to explain weather the effect of this SNP on gene expression of resistin, the disturbance of *retn* promoter region and the variation in this region may lead to increase resistin expression that may be a risk of T2DM.[33]

Actually, strong link was shown between rs34861192 G>A specially of AA genotype and *retn* gene expression. As we noticed later, this SNP present in promoter region of *retn* gene, many researchers focusing on promoter region of retn gene to indicate the SNP which related with the resistin expression, many of them indicate strong effect of these SNPs on circulating resistin levels. Authors (Osawa H, etal) recorded that there were genetic association between rs 1862513 and resistin levels that represent for Japanese cohort but this association was completely loss when conducted with influence of rs 34861192. The effect was particularly striking with methylation at cpg SNP within the regularity region of the gene.[34] One of the strong pieces of evidence of previous study was serous effect of rs34861192 refer to DNA methylation.

Actually, DNA methylation (DNA m) is an epigenetic modification that plays a role in the regulation of gene expression. About 23% of DNAm in blood cells has also been found to be heritable, and SNPs have been associated with differences in DNA m level. Furthermore, SNPs have been shown to influence mRNA levels through effects on DNA m. The rs34861192 SNPs in the retn promoter might thus influence DNA m around retn in monocytes and macrophages and thereby regulate resistin levels.[35]

CONCLUSION

There is strong connection between resistin with obesity, IR and T2DM through derangement of the signaling pathway of insulin**,** eventually this may lead to development of insulin resistance and T2DM. Polymorphisms of *retn* gene (rs34861192G>A) play an important role in development of T2DM, especially the level of mutant AA genotype that amplifies circulating level of resistin in T2DM patients. NG 023447 don't align with the pathogenesis of T2DM.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

Abbreviations: FBS-fasting blood sugar, T2DM-Type 2 dm diabetes mellitus, IR-insulin resistance, TLR4-Toll-like receptor 4, JNK- c-Jun N-terminal kinase, NF-κB – nuclear factor kappa B,MAPK-mitogen-activated protein kinase, NLRP-3-pyrin domain-containing protein, (NFkβ)-nuclear factor kappa-light-chain-enhancer of activated B cells, LRR-leucine rich repeat, TNF-α-tumor necrosis factor, IL-6-interleukine-6, IRS-insulin receptor substrate, TLR-toll like receptor, Δ-DCN- a variant of decurin.WAT-white adipose tissue.

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