

The Association of Resistin Polymorphism (3'UTR+62G→A) with Insulin Resistance and Hypertension at High Fat Diet Induced Type2 Diabetes in Rats: Experimental Study

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Abstract: Resistin, an adipocyte- and monocyte- derived cytokine that thought to be the missing link between obesity and diabetes. It also probably plays an important role in the development of insulin resistance. This factor considered to play an important role in the pathogenesis of type2 diabetes and insulin resistance-related hypertension. Recently, gene polymorphism is considered one of the most important factors that determine occurrence of diseases. Resistin gene polymorphism (UTR+62G>ASNP) may be a possible factor that link the hypertension with type 2DM. Aim: We aimed to investigate the correlation between resistin gene polymorphism (UTR+62G>A SNP) with serum resistin levels and if it is associated with insulin resistance-related hypertension in high fat diet- induced diabetes in rats (type 2 DM). Method: The current study was carried on 100 adult albino rats that have been divided into two groups. Group I: included 20 rats that were served as control group. Group II: that included 80 rats and they were given high fat diet for 16 weeks. At the end of the study, the blood pressure has been measured for both groups. Then the blood samples were collected to examine the serum glucose level, lipid profile level, serum resistin levels, the serum insulin levels and the allele and genotype frequencies of the single-nucleotide polymorphisms (UTR+62G>A SNP) in both groups. Insulin resistance was determined using HOMA-IR. The correlation of the gene variant to the hypertension, plasma resistin and insulin resistance have been investigated. Results: In the different diabetic groups, there was a significant association of the resistin A and G alleles of group IIb and group IIc when compared to controls ($X^2=12.21$, $X^2=46.88$, $P=0.000$) respectively. However, in group IIa the A and G allele were not significantly changed when compared to controls ($X^2=1.457$, $P=0.2$). Serum resistin levels were significantly increased in group IIa, group IIb and group IIc in comparison with the control group ($t=4.72$, $t=9.82$, and $t=17.64$ / $P=0.000$) respectively with a significant increase in group IIc than group IIb ($t=8.23$, $P=0.000$). There was a significant positive correlation between serum resistin and insulin levels ($r=0.939$, $P<0.0001$). In all groups, rats with GG genotypes carriers were found to have a significant increase of serum resistin, total cholesterol (TC), fasting blood glucose (FBG), serum insulin levels and HOMA index levels compared to AA genotype ($P<0.0001$). In conclusion: This study suggested that, the 62G/A polymorphism in resistin gene; genotype GG and G allele were significantly associated with increased serum resistin levels, hypercholesterolemia, increased insulin levels, and also associated with increased risk of insulin resistance and hypertension in obese diabetic rats.

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1. Introduction:

Resistin, an adipocyte- and monocyte-derived cytokine [1-3] that thought to be the missing link between obesity and diabetes [4]. It probably plays an important role in the development of insulin resistance with obesity [5,6]. Obesity by itself possibly accounts for 78% and 65% of essential hypertension in men and women, respectively, according to data from the Framingham Cohort [7]. Animal experiments and human studies have confirmed this causation and given insight into the mechanisms involved [8-12]. Hyperinsulinemia, hyperleptinemia, hypercortisolemia, renal dysfunction, altered vascular structure and function, enhanced sympathetic and renin-angiotensin system activity, and blunted natriuretic peptide activity

stand out as major contributory mechanisms to “obesity hypertension” [13-17]

Currently, it was found that serum resistin was positively correlated with several components of the metabolic syndrome, including hypertension in T2DM [18,19]. Also there are a growing volume of evidence demonstrates that a circulating resistin level and resistin gene single nucleotide polymorphisms are associated with the development of diabetes, hypertension, and atherosclerosis [20,21]. Furthermore, the allele and genotype frequencies of several SNPs of the resistin gene have been compared between patients with DM-2 and controls. No associations have been found in the vast majority of the studies [22-28]. An association has been found in two studies [20,29] i.e. in Chinese and Japanese populations.

The study from China reported that resistin gene polymorphism (+62G>A SNP) is an independent factor associated with systolic and diastolic blood pressures in type 2 diabetics. Diabetics with the GG genotype were found to have a higher prevalence of hypertension in this population. Authors of this article consider this polymorphism as an independent factor of type 2DM and arterial hypertension [29].

However, data from other study suggested that in a German Caucasian population the +62G→A polymorphism of the resistin gene is associated with hypertension but not with T2DM [30].

In the current study we investigated the association of resistin gene polymorphism (3'UTR +62G>A SNP) and hypertension that complicates high fat diet-induced type 2 diabetes in rats and also the association between this gene variants and blood glucose level, serum insulin level, lipid profile and insulin resistance has been investigated.

2. Experimental Protocol:

The current study was carried on 100 male adult albino rats (body weight, 180-200 gm) rats were housed under hygienic conditions, in the animal house of the Faculty of Medicine Zagazig University at 21°C–24°C in a 12 hr/12 hr light/dark cycle [31] for 16 weeks [32]. They were given free access to water and food. Rats were divided into two groups. **Group I:** included 20 rats that served as a control group; rats were fed standard rat chow that consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat (total 12.6 kJ/g) [33]. **Group II:** comprised of 80 rats those were given high fat diet (HFD) with 60% kcal% fat that consists of 16.45 proteins, 25.6% carbohydrate and 55.0% fat (total 23.4kJ/g) in the form of cotton seed oil added to the laboratory chow [33,34].

For all groups, body weight was recorded per week, and at the end of the study period.

At the end of the experimental period (the end of 16th week) after overnight fasting, at 8:00 a.m, blood pressure will be measured for both groups. Then the blood sample collected and divided into two divisions: a) serum was obtained by allowing the blood samples to clot at room temperature before centrifuging at approximately 3000 rpm for 15 minutes. The serum was stored at -20°C. to examine the resistin level, insulin level, fasting blood glucose (FBG) level and lipid profile. b) Whole blood samples were collected into EDTA-containing (1 g/L) tubes to study the allele and genotype frequencies of the single-nucleotide polymorphisms (+62G>A SNP) in both groups.

Measuring the blood pressures for rats:

After ketamine hydrochloride (45 mg/ kg b.wt.) intraperitoneal injection, the animals were implanted with cannulae (PE 50) inserted into the left common

carotid artery and the right jugular vein. The arterial cannula was connected to a pressure transducer fitted with F.C. 137 coupler connected to MD4- Oscillograph (Bioscience, Washington) [35].

Laboratory Analysis:

- 1- Serum resistin:** 9-The RD391016200R Rat Resistin ELISA Kits: is a sandwich enzyme immunoassay for the quantitative measurement of rat resistin (BioVendor-Laboratori medicina, U.S.A.) [36].
- 2- Determination of serum glucose level:** According to *Trinder* [37] using glucose enzymatic (GOD-PAP)-liquizyme Kits (Biotechnology, Egypt).
- 3- Determination of serum insulin level:** By a solid phase enzyme amplified sensitivity immunoassay according to *Starr et al.* [38] using KAP1251-INS-EASIA (Enzyme Amplified Sensitivity Immunoassay) Kits (BioSource Europe S.A., Belgium).
- 4- Determination of the Serum total cholesterol (TC):** Total Cholesterol (TC) TRTU 61218 kits: for enzymatic determination of total cholesterol (bioMerieux S.A., Lyon, France) according to *Allain et al.* [39]
- 5- Determination of the Serum high density lipoprotein cholesterol (HDL):** by enzymatic colorimetric method according to *Warnick et al.* [40], using Stanbio HDL-cholesterol procedure No. 0599 kits (Stanbio laboratory Inc., San Antonio, Texas, USA).
- 6- Determination of the Serum Triglyceride levels:** ESPAS SL kits: for enzymatic determination of triglycerides (Elttech S.A., Sees, France) according to *Naito et al.* [41].
- 7- Calculation of very low density lipoprotein cholesterol (VLDL) and Low density lipoprotein cholesterol:** According to *Friedewald et al.* [42] LDL was calculated as follows: $LDL = TC - HDL - TG/5$
- 8- HOMA-IR:** it was assessed by homeostasis model assessment (where $HOMA = (\text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting plasma glucose (mg/dl)}) / 405$) [43].

DNA Extraction:

DNA will be isolated using the DNA Isolation Kit. DNA will be extracted according to the method of *Bubbon*, [44].

The average DNA concentration was determined from absorbance at 260 nm. All samples DNA will be examined at a 260/280 nm absorbance ratio. The integrity of the DNA was checked by electrophoresis on 1.5 % agarose gel with an ethidium bromide.

Isolated DNA was used for determination of SNPs in the Resistin gene using polymerase chain reaction (PCR).

PCR method:

Detection details of SNP resistin +62G>A according to *Tan et al.* [29]

*Forward primer** 5'– GCC GAG ACC ACA TGT CAC T – 3'

*Reverse primer** 5'– CCT CCG GGC CTA CTA AAG AA – 3'

PCR Conditions: 96°C/2min 1x/ 94°C/30s 35x /54,3°C/30s 35x/ 72°C/30s 35x /72°C/5min 1x. in a thermal cycler (a Perkin Elmer 4800 thermal cycler (PTC-100 machine, MJ Research, Inc., Watertown, Mass. USA).

Using Restriction Enzyme of BseR I ° /RFLP conditions 37°C/24h [45] °Fermentas Life Sciences, Lithuania PCR and RFLP products were detected by electrophoresis on 2 % agarose gel with 1 % ethidium bromide.

Gel electrophoresis with 1.5 % agarose gel and Ethidium bromide (5mg/ml) for PCR-digested products isolation. Sub-marine gel electrophoresis was used, (Pharmacia Biotech by SEMKO AB, Sweden) using submarine chamber (Maxicell, EC 360 M-E-C apparatus Cooperation ST. Petersburg. Florida USA).

Sample Preparation and Loading:

Each digested product sample prepared and electrophoresed on agarose as follow: To every 5 µl of digested DNA product, 5 µl of 6X gel loading dye (prepared by bromophenol blue 0.25% and sucrose 40% in 50 mM EDTA) was added and then the gel was visualized under UV transilluminator with 100 base pair ladder and photographed .

Statistical Analysis:

The data obtained in the present study were expressed as mean ± SD for quantitative variables and statistically analyzed by using SPSS program (version 17 for windows) (SPSS Inc. Chicago, IL, USA). Analysis of variance (ANOVA) was used to compare the results of all examined cases in all studied groups within group comparisons. The differences between mean values for each element were tested by student's "t" test. The Hardy-Weinberg equilibrium or odds ratio (OR) and 95% confidence interval (CI) for the presence of obesity and insulin resistance within the resistin genotypes were analyzed by using the chi square χ^2 test. P value <0.05 was considered statistically significant [46].

3. Results:

Rats of then divided according to their clinical and laboratory data into:

Group I: control group (their final body weight was 240.72±1.69 g, their blood glucose was 74.6 ±7.70 mg/dl their arterial blood pressure was S=118±9.19; D=79±7.38 and their HOMA-IR value was 3.42±0.75.

Group IIa: Contain of 12 obese rats with normal blood glucose and blood pressure and insulin resistance (their final body weight was 277.88±3.15g, their blood glucose was 81.80±6.83 mg/dl, their arterial blood pressure was S=119±7.38/D=81±5.68 and their HOMA-IR value was 4.14±0.72.

Group IIb: included 24 obese diabetic rats with insulin resistance but with normal blood pressure (their final body weight was 280.47±2.64g, their blood glucose was 200.42±38.65 mg/dl, their arterial blood pressure was S=118±7.89/D=81±5.68 and their HOMA-IR value was 23.34±6.79.

Group IIc: included 44 obese diabetic rats with insulin resistance and hypertension. (Their final body weight was 281.96±2.80g, their mean blood glucose was 275.37±30.03 mg/dl, their arterial blood pressure was S=166±10.75/D=120±12.47 and their HOMA-IR value was 41.96±9.75)

After restriction digestion with BseR I °, the GG genotype resulted in 249bp fragments, AA genotype resulted in 238 and 11bp fragments, the G/A genotype resulted in 3 fragments of 249,238 and 11bp. Genotype and alleles frequencies for MTHFR are presented in *table (2) and figure (1).*

In controls (*GroupI*): the A allele frequency was 90%, the G allele frequency was 10%. The detected level of heterozygosity AG was in 20%, and the AA genotype was the most common in controls (80%).

In Group IIa: the homozygous AA was found in 7 rats (58.33%), the heterozygous AG in 5 rats (41.66%). Chi square (χ^2) for group II=1.742 and there was significant association when compared with the controls (P= 0.001).

A allele frequency was 79.16 % and G allele frequency was 20.83%, when compared with controls there was non-significant association ($\chi^2=1.457$, P=0.200) (OR=2.368 with 95% CI of 0.568-9.871).

In Group IIb: The AA pattern was observed in 7 rats (29.16 %), the AG in 13 rats (54.16%) and the GG pattern in 4 rat (16.66%), there was a significant association when compared with the controls ($\chi^2=12.02$, P=0.001). A allele frequency was 56.25% and the G allele frequency is 43.75 % ,with significant association when compared to controls ($\chi^2=12.21$, P=0.000) (OR=7.00 with 95% CI of 2.151-22.779).

In Group IIc: the AA pattern frequency was 13.63%, the AG was 22.72% and the GG pattern was 63.63 %, there was a significant association when compared with the controls ($\chi^2=30.39$, P=0.000). A allele frequency was 25% and the G allele frequency is 75%, with significant association when compared to controls ($\chi^2=46.88$, P= 0.000) (OR= 27.00 with 95% CI of 8.634 -84.433).

In all studied groups, the AA pattern was found in 36 rats (36%), the AG in 32 rats (32%) and the GG

pattern in 32 rodents (32%). A allele frequency was 52% and the G allele frequency is 48 % (Table 2).

The relation between plasma resistin with genotype variants of +62G→A polymorphism of resistin gene:

Mean±SD of serum resistin levels related to different groups listed in table (1) and figure (2). The different genotypes in all groups showed different means of serum resistin levels listed in table (3). ANOVA test revealed a significant difference of the mean values of serum resistin level among different studied groups ($F=141.481$, $P=0.000$). Serum resistin levels were significantly increased in group IIa, group IIb and group IIc than control group ($t=4.72$, $t=9.82$, and $t=17.64$, $P=0.000$) respectively with a significant increase in group IIc than group IIb ($t=8.23$, $P=0.000$). There was a significant positive correlation between serum resistin and insulin levels ($r=0.939$, $P<0.0001$) Figure (3).

Table (4): In all groups, rats with GG, GA genotypes have a significant increase of serum resistin, TC, FBG, serum insulin levels and HOMA index levels compared to AA genotype, with more significant increase in GG genotype.

Data of lipid profile was listed in table (1). Regarding TC, there was a significant increase in group IIc, group IIb and group IIa than controls ($t=6.29$, $t=30.08$ and $t=18.1$, $P=0.000$) respectively, with non-significant difference between group IIa and group IIb ($t=-2.721$, $P=0.053$).

HDL-C levels showed significant decrease in group IIa, group IIb and group IIc than controls ($t=23.286$, $t=6.907$, $t=10.87$, $P<0.000$) respectively, with non-significant change among diseased rat groups ($P>0.05$). There was a significant association between lipid profile and the genotype pattern of the resistin gene that serum TC levels were significant increase in GG genotype more than AG and AA ($t=3.354$, $P=0.020$, $t=30.420$, $P=0.000$) respectively.

Regarding FBG levels, there was a significant increase in group IIb and group IIc and when compared to controls ($t=7.91$, 18.1 , $P<0.0001$) respectively, with significant increase in group IIc more than group IIb ($t=9.77$, $P=0.000$) and non-significant difference between group IIa and controls ($t=1.90$, $P=0.129$). There was significant association between FBG and the genotype pattern of the resistin, FBG levels were significantly increased in GG genotype when compared to AG and AA genotypes ($t=6.142$, $P=0.002$, $t=22.532$, $P<0.0001$) respectively.

Serum insulin levels revealed significant increase in group IIb and group IIc when compared to controls ($t=10.07$, $t=13.37$, $P<0.0001$) respectively, with significant increase in group IIc more than group IIb ($t=5.56$, $P<0.001$) and non-significant difference between group IIa and controls ($t=-1.34$, $P=0.251$).

There was significant association between serum insulin levels and the genotype pattern of the resistin gene, there was significant increase of serum insulin in GG genotype when compared to AG and AA genotypes ($t=4.53$, $P=0.006$, $t=18.23$, $P<0.0001$) respectively.

Concerning HOMA index levels, there was a significant increase in group IIb and group IIc when compared to controls ($t=7.2$, 10.9 , $P<0.0001$) respectively, with significant increase in group IIc more than group IIb ($t=6.909$, $P=0.000$) and non-significant difference between group IIa and controls ($t=-1.51$, $P=0.205$). There was significant association between HOMA index and the genotype pattern of the resistin, HOMA levels were significantly increased in GG genotype when compared to AG and AA genotypes ($t=5.081$, $P=0.004$, $t=12.694$, $P<0.0001$) respectively.

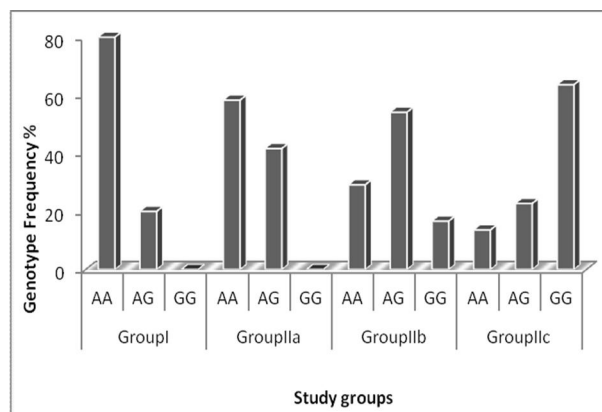


Fig.(1) :Frequency of resistin gene genotypes in all studied groups.

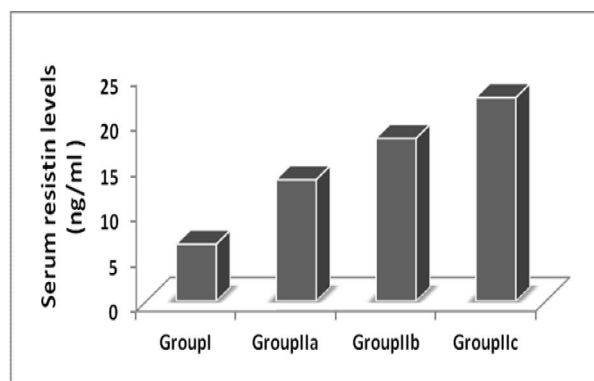


Fig. (2). Mean of serum resistin levels in all studied groups.

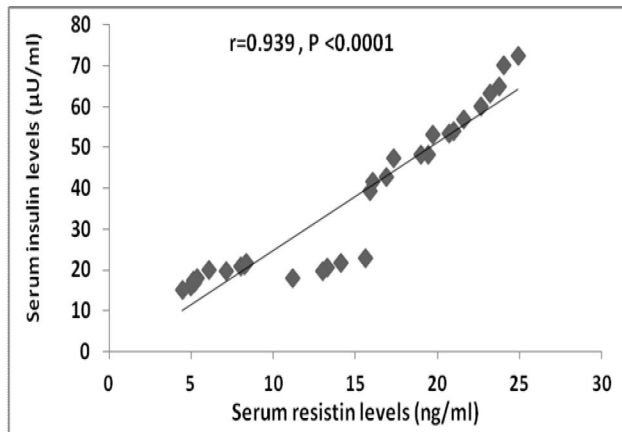


Fig. (3): Correlation between of serum resistin and insulin levels in all studied groups.

Table (1): Comparison of clinical and laboratory data of the all studied groups (mean \pm SD and range, with anova test (F- test).

	Group I	Group IIa	Group IIb	Group IIc	F value P value
Body Weight (gm)	240.72 \pm 1.69 238.32-243.32	277.88 \pm 3.15 270.53-281.24	280.47 \pm 2.64 276.67-286.30	281.96 \pm 2.80 277.35-285.21	F=566.759 P=0.000
Blood pressure (mmHg)	S=118 \pm 9.19 D=79 \pm 7.38	S=119 \pm 7.38 D=81 \pm 5.68	S=118 \pm 7.89 D=81 \pm 5.68	S=166 \pm 10.75 D=120 \pm 12.47	F=71.779/P=0.000 F=57.462/P=0.000
Resistin (ng/ml)	6.29 \pm 1.49 4.46-8.36	13.43 \pm 1.60 11.20-15.60	18.0 \pm 1.92 15.89-20.71	22.51 \pm 1.91 18.99-24.90	F=141.481 P=0.000
TC (mg/dl)	117.7 \pm 6.94 106.0-127.0	212.2 \pm 33.04 165.0-240.0	252.0 \pm 8.06 242.0-266.0	272.6 \pm 22.14 246.0-305.0	F=127.892 P=0.000
TG (mg/dl)	51.10 \pm 5.48 42.0-60.0	100.8 \pm 14.53 87.0-125.0	137.14 \pm 9.13 126.0-152.0	160.5 \pm 24.61 128.0-201.0	F=89.674 P=0.000
HDL-C(mg/dl)	65.8 \pm 10.58 45.0-76.0	36.8 \pm 4.14 32.0-42.0	37.0 \pm 4.72 30.0-42.0	34.12 \pm 3.68 30.0-41.0	F=40.088 P=0.000
LDL-C(mg/dl)	41.72 \pm 15.5 21.60-70.0	153.44 \pm 38.14 96.60-183.0	187.57 \pm 10.9 174.8-205.6	218.9 \pm 34.03 179.4-288.0	F=84.074 P=0.000
FBG (mg/dl)	74.6 \pm 7.70 64.0-87.0	81.80 \pm 6.83 74.0-91.0	200.42 \pm 38.65 130.0-240.0	275.37 \pm 30.03 223.0-310.0	F=119.898 P=0.000
Insulin (μU/ml)	18.66 \pm 2.28 15.23-21.61	20.59 \pm 1.88 17.91-22.91	46.56 \pm 5.54 39.31-53.46	61.31 \pm 8.17 48.21-72.64	F=122.28 P=0.000
HOMA index	3.42 \pm 0.75 2.36-4.42	4.14 \pm 0.72 3.26-5.14	23.34 \pm 6.79 12.57-31.60	41.96 \pm 9.75 26.35-54.88	F=71.768 P=0.000

Table (2): Allele and genotype frequencies for resistin TR +64G→A polymorphism in all studied groups

Groups	Genotype frequency n (%)			Allele frequency n (%)	
	AA	AG	GG	A	G
Group I	16 (80%)	4 (20%)	0 (0%)	36 (90%)	4 (10%)
GroupIIa	7(58.33%)	5 (41.66 %)	0 (0%)	19 (79.16%)	5(20.83%)
GroupIIb	7 (29.16%)	13 (54.16%)	4 (16.66%)	27 (56.25%)	21(43.75%)
GroupIIc	6 (13.63%)	10 (22.72%)	8 (63.63%)	22 (25 %)	66(57%)
All groups	36 (36%)	32 (32%)	32 (32%)	104 (52%)	96 (48%)

Table (3): Chi square and OR (95% CI) of resistin genotypes and allele frequency in all studied groups.

Groups	Resistin gene Genotype	A and G Allele frequency	OR of A and G allele frequency 95% CI

Control group Vs Group II	$\chi^2=1.742$ $P=0.18$	$\chi^2=1.457$ $P=0.20$	2.368 0.568-9.871
Control group Vs Group III	$\chi^2=12.02$ $P=0.002$	$\chi^2=12.21$ $P=0.000$	7.00 2.151-22.779
Control group Vs Group IV	$\chi^2=30.39$ $P=0.000$	$\chi^2=46.88$ $P=0.000$	27.0 8.634-84.433

Table (4): Comparison of some biochemical parameters and different genotypes in all studied groups

	AA n=14	AG n=10	GG n=6	t- value	P value
Serum resistin (ng/ml)					
Range	4.46-18.99	8.23-21.61	20.71-24.90	-6.019*	P=0.000
Mean \pm SD	9.68 \pm 5.03	16.14 \pm 4.83	23.20 \pm 1.43	-5.431**	P=0.003
				34.802***	P=0.000
TC (mg/dl)					
Range	106.0-246.0	125.0- 258.0	266.0-305.0	-5.787*	P=0.000
Mean \pm SD	160.92 \pm 58.6	223.4 \pm 52.0	282.6 \pm 16.94	-3.354**	P=0.02
				-30.42***	P=0.000
FBG (mg/dl)					
Range	64.0-223.0	85.0- 266.0	240.0- 310.0	-4.44*	P=0.002
Mean \pm SD	94.78 \pm 46.76	172.5 \pm 75.37	284.83 \pm 25.6	-6.142**	P=0.002
				22.532***	P=0.000
Serum insulin (μ U/ml)					
Range	15.23-48.21	20.81-56.92	53.46-72.64	-4.211*	P=0.002
Mean \pm SD	23.7064 \pm 10.7	38.94 \pm 15.3	64.12 \pm 6.91	-4.530**	P=0.006
				18.232***	P=0.000
HOMA index					
Range	2.36-26.35	4.41-37.18	31.60 - 54.88	-3.741*	P=0.005
Mean \pm SD	6.61 \pm 7.13	19.03 \pm 13.12	45.18 \pm 8.46	-5.081**	P=0.004
				12.694***	P=0.000

*referred to t- value of comparing AA with AG, **referred to t- value of comparing AG with GG while

***referred to t-value of comparing AA with GG of all patient groups.

4. Discussion:

Current evidence from animal models indicates that a reduction in functional resistin protein (via gene knockout, anti-resistin immunoglobulin, and dominant inhibition) can lower blood glucose levels and improve insulin sensitivity [4,47,48]. In addition, elevated levels of serum resistin in diabetic db/db mice implicate resistin as a key mediator between adiposity and type2 diabetes [4]. Therefore, the role of resistin as an adipocyte secreted cytokine inducing insulin resistance appears to be established in rodents. In humans, circulating resistin levels are higher among individuals with type 2 diabetes than among apparently healthy individuals [18,49,50].

Disordered fat storage, mobilization, and failure of fat cell proliferation may result in ectopic fat storage, insulin resistance, and type 2 diabetes [51,52]. As resistin plays a role in adipocyte differentiation [53]. So in the current study we proposed that there are a relationship between the 3'UTR+62G→A variant and resistin gene expression in addition that this variant may contribute to the pathogenesis of insulin resistance and type2 diabetes through influencing the adipocyte differentiation and maturation. Moreover, the association of hypertension with the resistin gene polymorphism was expected, as it is well known that there are strong relationships between obesity, insulin resistance and hypertension [54,55].

The results of the current study revealed that resistin gene 3'UTR +62G→A genotype and alleles showed significant association to obesity and diabetes without and with hypertension (GroupIIb and groupIIc) respectively when compared to controls group with significant increase in GG genotype and G allele in these two groups than controls. Furthermore, rats with GG, GA genotypes have a significant increase of serum resistin, TC, FBG, serum insulin levels and HOMA index levels compared to AA genotype, with more significant increase in GG genotype. These results were in consistence with the results of *Tan et al.* [29], who indicated that the +62A variant of the resistin gene 3'UTR is associated with reduced risk for the development of diabetes and hypertension. In addition, plasma resistin level was associated with dyslipidaemia, serum insulin concentration and obesity.

The results of *Asano et al.* [56] indicated that the rs34861192 and rs3745368 {Other name for resistin gene 3'UTR+62G→A polymorphism [57]} were significantly associated with the resistin level and consider those polymorphisms of resistin as robust and independent determinants of plasma resistin concentration in the studied population.

However, others indicated that obese patients with the minor A allele in position +62 of the resistin

gene had higher cholesterol levels than OB patients with the G/G genotype in the same position [58].

Regarding the relation between the resistin polymorphism and blood pressure our finding suggested significant association between the hypertension complicating obese diabetic group and the resistin gene polymorphism. How could a resistin gene polymorphism be associated with hypertension? It may be through the role of resistin in promotion of endothelin-1 release from endothelial cells [59], a potent vasoconstrictor involved in the pathogenesis of hypertension [60], and furthermore, the resistin like molecule (RELM) has been shown to have vasoconstrictive properties [61]. This could be confirmed also by the indicated association between the +62G→A variant and plasma resistin levels. These finding have been also shown for the (537A>C, 420C>G, 638G>A, and 358G>A) variants [62,63]. Moreover, the results of the current study also provided a strong link between insulin resistance and hypertension, suggesting that either insulin resistance or an adipocyte related cytokine might play a role in the pathogenesis of hypertension. There have been reports providing evidence that insulin resistance plays some part in blood pressure regulation [64,65]. The cause behind the association between insulin resistance and hypertension remains may be due to impairment of the insulin-mediated vasodilatation in an insulin-resistant state [64-66]. Moreover, it is possible that insulin resistance not only affects glucose metabolism, but also vasodilatation, eventually leading to hypertension. Various thiazolidinediones have been shown to have antihypertensive properties in experimental rats [67-69] and in human subjects [66,70,71]. *Nolan et al.* [70] demonstrated that troglitazone resulted in a significant reduction in both systolic and diastolic blood pressures, as assessed by 24-h ambulatory monitoring, in normotensive insulin-resistant human subjects accompanied by improved glucose tolerance and enhanced insulin sensitivity.

Gouni-Berthold et al. [30] found an association between the presence of the 3'UTR +62G→A polymorphism of the resistin gene and hypertension. However, they found that the +62G→A polymorphism did not affect serum resistin level. And as there were no insulin sensitivity measurements in that study, it couldn't be excluded that resistin is associated with hypertension through an increase in insulin resistance.

Possible relationships between different resistin gene polymorphisms and DM-2 have been extensively investigated, mostly with negative results [22-27,72]. However, other studies have found an association between a resistin gene polymorphism and DM-2, one in a Chinese [27], Japanese [28] population, Finnish [21] and Caucasians [24]. Furthermore, a differential effect of a resistin gene SNP and BP between DM-2

and controls was also found by *Conneely et al.* [73]. In specific, the authors found an association between increased systolic BP and the +1084G>A polymorphism from the 3'UTR in the control but not in the DM-2 group. The authors suggested that the contradictory findings between the diabetic and the control group might be the result of interactions with other genes or environmental factors that differ between the two groups.

The wide discrepancy between the result of association of the resistin gene polymorphism with obesity, Diabetes, insulin resistance and hypertension may be due to ethnic differences as well as variable gene-environment interactions influencing the phenotypic expression of the variant [26]. Furthermore, it cannot be ruled out that the associations found in the present study are because of another polymorphism in linkage disequilibrium with the variant studied [74].

Conclusion

The present study stated that, the 3'UTR +62G→A polymorphism of the resistin gene is associated with insulin-resistance and increased blood pressure in diabetic obese rats. Our data would support multiple further studies on the functional role of resistin in human and can be considered as a step in human further studies which links obesity to diabetes insulin resistance and hypertension, additional researches are needed to explore the role of resistin, resistin receptors and their mechanisms of action in Insulin resistance, obesity and hypertension. Moreover, studying and analysis of more resistin gene variants with larger sample of subjects are required to clarify the role of the resistin gene polymorphism in the patho-physiology of T2DM and insulin resistance related hypertension and confirm the significance and non-significance of our results.

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