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RESEARCH ARTICLE

CIRCULATING LEVELS OF RESISTIN AND +299 (G>A) RESISTIN GENE POLYMORPHISM IN TYPE 2 DIABETES MELLITUS, A STUDY FROM NORTH INDIA

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ABSTRACT

Introduction: Obesity is a risk factor for diabetes mellitus. Resistin, an adipokine explains the link between obesity and diabetes. The present study was conducted to explore the association between resistin gene polymorphism and type 2 diabetes mellitus (DM).
Study Design: It was a case control study consisted of 90 Indian subjects divided into three groups as cases (n=30), obese person without diabetes (n=30) and healthy controls (n=30). Following parameters were measured: age, body mass index (BMI), waist hip ratio (WHR), lipid profile, plasma glucose, insulin, homeostatic model assessment for insulin resistance (HOMA-IR) and resistin. Comparison of data was done by using one way ANOVA and odd's ratio was determined to calculate the risk.
Results: Serum resistin was significantly higher in cases of type 2 diabetes with +299 (G>A) genotypes. Resistin gene polymorphism +299 (G>A) is a significant risk for type 2 diabetes.
Conclusion: Resistin gene polymorphism +299(G>A) has an association with diabetes and increase the susceptibility of type 2 DM.

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INTRODUCTION

Diabetes mellitus (DM), long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century. It is the most common non-communicable disease worldwide and the fourth to fifth leading cause of death in developed countries (Kahn *et al*., 2005) It is basically a heterogenous group of metabolic disorder characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism (WHO, 1999). Insulin resistance is an early and strong determinant of type-2 diabetes mellitus (T2DM) (Lee *et al*., 2010). Resistin, a hormone produced by white adipose tissues is related to both insulin resistance and obesity (Steppan *et al*., 2001). It plays role in regulation of energy, glucose and lipid homeostasis (Steppan *et al*., 2002). It also maintains the blood glucose level by modulating insulin action (Patel *et al*., 2003; Banerjee *et al*., 2004). Resistin, by increasing the expression of various pro inflammatory cytokines like IL-1, 6, 12 and TNF-α cause insulin resistance (Wallen *et al*., 2005). The gene for resistin (RETN) is located on chromosome 19p13.3 (Steppan *et al*., 2001). Several single nucleotide polymorphisms (SNP) have been described in RETN promoter, intron and 3' untranslated region (Pizzuti *et al*., 2002). One of the polymorphism of resistin gene is 299(G>A) which in a recent study has shown a strong association with type 2 diabetes in Caucasians (Suriyaprom *et al*., 2009). Clearly, more studies are required to clinch association between serum resistin level and resistin gene polymorphism with type 2diabetes (Amos 2010). Keeping the above in view, the present case-control study was carried out with objectives: (a) to estimate serum resistin levels in type 2 diabetes mellitus and (b) to find the status of +299(G>A) resistin gene polymorphism in type 2 diabetes.

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MATERIALS AND METHODS

Study area, design and participants

The study was conducted in Department of Biochemistry, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi. It was a case control study comprised of total 90 subjects. The study was conducted from December 2009 to August 2010. The subjects were allocated into three groups as: (a) Group I included 30 cases of type 2 diabetes mellitus based

upon American Diabetic Association criteria (Henrikesan *et al*., 1994) who were recruited from Medicine OPD and Diabetic Clinic, Lok Nayak Hospital, New Delhi (b) Group II included 30 obese volunteers who had no history of diabetes as controls matched with respect to age and sex, and (c) Group III included 30 normal healthy volunteers as controls matched with respect to age and sex. Patients of type 1 and secondary diabetes were excluded from the study. Written informed consent was taken from all the subjects. The study was approved by ethical committee of the college. A detailed physical examination was done. Blood pressure (BP), waist-hip ratio (WHR), height and weight of all the subjects were taken. Body mass index (BMI) was calculated as weight (kg)/height (meter)^2. Fasting blood sample was obtained from all the subjects. Two ml blood was collected in EDTA vial for genotyping, 1 ml was collected in fluoride vial for plasma glucose estimation and 3 ml was taken in plain vial for other parameters.

Method and Calculation

Serum resistin was measured by sandwich ELISA provided by Bio Vendor, Germany. Serum insulin was estimated by electrochemiluminescence assay on ROCHE Elecsys 2010 using kit from Roche Diagnostic, USA. Estimation of blood glucose, blood urea, serum creatinine and lipids were done by enzymatic methods on Olympus AU 400 using commercially available kits. Homeostatic Model Assessment for insulin resistance (HOMA-IR) was calculated by using HOMA model of Mathews and colleagues which uses fasting plasma glucose and insulin concentration (Mathews *et al*., 1985). HOMA-IR= {Serum Insulin (µIU/mL) X Fasting Plasma Glucose (mg %)} / 405.

Polymerase chain reaction

DNA was extracted from whole blood using MB 504 Hi Pura ATM blood genomic DNA miniprep purification spin kit from Hi Media Laboratories. The required region of resistin gene from the genomic DNA was amplified by PCR using primers forward 5' GAG AGG ATC CAG GAG GTC 3' and reverse 5' GTG AGA CCA AAC GGT CCC TG 3' as described by Kunnari *et al* (Kunnari 2005). The amplicons were digested with Alu I restriction endonuclease from Fermantas, Thermoscientific USA. The digested products were resolved using electrophoresis in 2% agarose gel.

Data analysis

Statistical analysis was done by SPSS 17. One way ANOVA, Tukey HSD post-hoc test and Kruskall Wallis test were used to analyse the data. The relationship between +299 G>A resistin gene polymorphism and type 2 diabetes mellitus was determined using odd's ratio.

RESULTS

Comparison of anthropometric and biochemical parameters were seen in the three groups (healthy control, obese control group and patients of diabetes mellitus) using one way ANOVA and Tukey post hoc test (TABLE 1). There was no significant difference in the age in three groups suggesting all the three groups were age and sex matched. BMI and WHR were significantly higher in obese group and cases as compared to healthy group and BMI was also significantly higher from obese group controls. Among the lipid profile parameters, HDL was significantly lower and TAG were significantly higher in cases as compare to healthy and obese

Table 1. Comparison of anthropometric and biochemical parameters among healthy controls, obese group and cases

Variables	Healthy controls (N=30) Mean ± SD	Obese controls (N=30) Mean ± SD	Cases (N=30) Mean ± SD
Age (years)	46.07 ± 8.80	47.27 ± 7.50	48.30 ± 7.47
BMI (kg/m2)	23.51 ± 1.53	34.44 ± 3.09*	26.43 ± 2.64*§
WHR	0.93 ± 0.04	0.97 ± 0.05*	0.98 ± 0.06*
Serum Total CHOL (mg/dl)	137.63 ± 19.47	176.27 ± 35.93*	156.43 ± 36.22§
Serum HDL-C (mg/dl)	41.00 ± 12.00	37.40 ± 8.56	29.67 ± 9.36*§
Serum LDL-C (mg/dl)	97.80 ± 25.33	131.13 ± 32.92*	112.00± 31.50§
Serum TAG (mg/dl)	105.30 ± 28.28	163.43 ± 48.50*	201.37 ± 73.70*§
Serum FPG (mg/dl)	92.87 ±14.39	125.87 ±32.53*	178.73 ±74.74*§
Serum Insulin	10.72 ±4.72	35.06 ±17.50*	17.21 ±13.71§
HOMA-IR	2.50 ± 1.27	11.41 ±8.14*	7.46 ±7.72*
Serum Resistin (ng/mL)	13.13±5.79	15.38±5.41	23.80±13.25*§

*p < 0.05 in comparison to healthy control.
§ p < 0.05 in comparison to obese control by one way ANOVA with Tukey HSD post-hoc test.

Table 2. Distribution of GG v/s GA+AA genotypes in cases & healthy controls

Genotypes	Cases N=30 (%)	Healthy Controls N=30 (%)	OR (95% CI)	p value
GG	13 (43.3)	21 (70.0)	3.1	0.04*
GA+AA	17 (56.7)	09 (30.0)	(1.1 – 8.8)	

*Significant (p < 0.05)

Table 3. Distribution of GG v/s GA+AA genotypes in healthy controls and obese controls without diabetes

Genotypes	Obese Controls N=30 (%)	Healthy Controls N=30 (%)	OR (95% CI)	p value
GG	20 (66.7)	21 (70.0)	0.85	0.77
GA+AA	10 (33.3)	09 (30.0)	(0.28 –2.54)	

Table 4. Comparison of anthropometric and biochemical parameters according to GG vs. GA+AA genotypes in cases, obese control and healthy control groups

Groups	Parameter	GG(13)	GA+ AA(17)	p value
Cases (N = 30)	BMI (Mean ± SD)	26.56 ± 2.30	26.37 ± 2.30	0.23
	WHR (Mean ± SD)	0.99 ± 0.647	0.97 ± 0.069	0.86
	HOMA-IR (Mean ± SD)	6.19 ± 4.94	8.43 ± 9.34	0.25
	Resistin (Mean ± SD)	17.64± 7.83	28.51± 14.75	0.02*
	Parameter	GG (20)	GA+AA(10)	
Obese Controls (N = 30)	BMI (Mean ± SD)	33.41 ± 2.48	34.81 ± 3.25	0.40
	WHR (Mean ± SD)	0.96 ± 0.05	0.98 ± 0.60	0.62
	HOMA-IR (Mean ± SD)	12.28 ± 9.56	9.68 ± 3.91	0.17
	Resistin (Mean ± SD)	14.67± 6.07	16.81 ± 3.59	0.31
	Parameter	GG (21)	GA+AA(9)	
Healthy controls (N = 30)	BMI (Mean ± SD)	23.14 ± 1.68	23.63 ± 1.51	0.79
	WHR (Mean ± SD)	0.95 ± 0.25	0.92 ± 0.04	0.06
	HOMA-IR (Mean ± SD)	2.46 ± 1.14	2.59 ± 1.62	0.72
	Resistin (Mean ± SD)	12.88± 5.86	13.7± 5.94	0.73

*Significant ($p < 0.05$)

control group suggesting dyslipidemia in type 2 diabetes mellitus. There was a steady and significant increase in blood glucose from healthy controls to obese group and then to cases. HOMA-IR was significantly higher in obese group and person with diabetes group in comparison to healthy control. Serum resistin was also significantly higher in cases as compared to healthy and obese group. Distribution of resistin gene genotypes (Healthy GG & Mutant GA+AA) were seen in case & healthy control (TABLE 2). The mutant forms of gene were significantly more in cases (56.7%) as compare to in healthy control (30.0%) (Odd's ratio = 3.1, 95% CI = 1.1 – 8.8, p = 0.04). The resistin gene genotypes distribution was also seen in the obese controls and healthy control (TABLE 3). The mutant forms of gene were more in obese controls (33.3%) as compare to in healthy control (30.0%) but it was not significant (Odd's ratio = 0.85, 95% CI = 0.28 – 2.54, p = 0.77). Distribution of anthropometric and biochemical parameters was also seen in the two genotypes (GG vs. GA+AA) in three groups (Table 4). Serum resistin was significantly (p=0.02) more in mutant genotype (GA+AA) as compare to wild type genotype (GG) of cases.

DISCUSSION

Developing countries like India have had the maximum increase in the cases of type 2 diabetes in the last few years. This increase in prevalence of type 2 diabetes is explained by a similarly sharp rise in obesity, which is primarily a risk factor for type 2 diabetes (Genuth *et al*., 2003). The hormone resistin links obesity to diabetes, so it is considered a hot topic of obesity and diabetes research. SNP +299 (G>A) resistin gene polymorphism has been studied for the association of circulating resistin levels with obesity and diabetes mellitus risk but prevalence of this polymorphism and its association with type 2 diabetes risk in Indian population is not known. So the present study was conducted with the aim to study the occurrence of resistin gene polymorphism in type 2 diabetes mellitus in Indian population. The mean age of cases was 48.30±7.47 years in present study. This was in agreement of another study conducted in New Delhi which found average age of patients of type 2diabetes was 52.13±9.9 years (Kanakamani *et al*., 2010). BMI and WHR of cases in the present study were found to be 26.43±2.64 kg/m^2 and 0.98 ± 0.06 respectively. The similar findings were obtained in other studies (Kanakamani, 2010; Schmidt, 1992). Dyslipidemia in diabetes is characterised by elevated triglycerides and reduced HDL-C. Low HDL-C is independently associated with resistance to insulin mediated glucose uptake (Tangvarasittichai, 2010). Similar kind of lipid profile picture was found in our study with mean HDL of 29.67 mg/dL and mean TG of 201.37 mg/dL suggesting dyslipidemia in cases of type2 diabetes. Insulin resistance was calculated using HOMA-IR and it was found to be significantly higher in obese controls and persons with diabetes in comparison to healthy control. Several studies have reported increased insulin resistance in subjects with type 2 diabetes mellitus when insulin resistance was assessed using HOMA-IR (Hanefeld *et al*., 2003; Melchionda *et al*., 2002).

Mean value of plasma glucose was more in cases as compare to obese and healthy group but serum insulin and HOMA-IR was maximumin obese group. It was less in cases of type 2 diabetes which may be due to reason that patients were already on treatment of diabetes and may be due to β cell dysfunction.
In our study the mean value (± SD) of serum resistin (ng/mL) was significantly higher in cases (23.80± 13.25) as compared to controls (p=0.00). Studies on the plasma resistin level in type 2diabetes have described conflicting results. There are studies that have reported higher plasma resistin concentrations in subjects with type 2 diabetes (Al Daghri *et al*.,2005; Dullaart *et al*., 2007; Fujinami *et al*., 2004; Hasegawa *et al*., 2005) whereas there is an almost equal number of studies in which no such difference has been observed (Chen *et al*., 2006; Fehmann., 2002; Yaturu, 2006). Table 2 shows that mutant form GA/AA genotype was significantly higher in cases than healthy control (p=0.04) in present study. Similar finding was seen in a study on Thai population that resistin gene +299 (G>A) polymorphism with GA/AA genotype was significantly higher (p=0.04) in persons with type 2 diabetes as compared to controls (Suriyaprom *et al*., 2009). On the contrary, a study done by Ochi *et al* on Japanese population reported that a frequent single nucleotide polymorphism (SNP) +299 (G>A) in this gene is not associated with type 2 diabetes (Ochi *et al*., 2003). The association of + 299 (G>A) polymorphism with

type 2 diabetes and obesity was observed using odd's ratio (Table 2 and 3). The gene polymorphism was significantly associated with diabetes (odd's ratio=3.1, p=0.04) but not with obesity suggesting role of resistin polymorphism in diabetes but not in obesity as thought earlier. In another study similar association of resistin with diabetes was seen with odds ratio of 1.93 (p=0.042) (Suriyaprom *et al*., 2009). No significant difference was found in BMI and WHR in carriers of GG and GA/AA in present study among cases, obese controls and healthy controls. Similarly, in a study by Suriyaprom, it was found that with regard to +299(G>A) polymorphism in type 2 diabetes subjects, there were no differences in anthropometric variables (BMI and WHR) (Suriyaprom *et al*., 2009). According to table 4 resistin gene polymorphism +299(G>A) was not showing any effect on level of resistin in healthy and obese without diabetes group. But it has definitely affected resistin levels in persons with type 2 diabetes with p=0.02 suggesting the association of this polymorphism in type 2 diabetes. Thus this differential expression of resistin gene in persons with diabetes may be due to the effect of other genes and environmental factors that contribute to diabetes but the interaction needs to be explored.

Conclusion

So we conclude that despite being polygenic / multifactorial disease, the genes that contribute to pathogenesis of type 2 diabetes interact with resistin gene and thereby its expression in mutant (GA+AA) genotype. There is a significant association between +299 (G>A) polymorphism and Type 2 diabetes mellitus risk but further studies are needed to fully clarify the role of the resistin gene in type 2 diabetes.

Conflict of interest

Authors declare that there is no conflict of interest.

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