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INTERLEUKIN-6(IL-6) GENE POLYMORPHISMS ASSOCIATED WITH TYPE 2 DIABETES MELLITUS IN KERALA POPULATION

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Abstract
Inflammatory mechanisms play a key role in the pathogenesis of type 2 diabetes. Individuals who progress to type 2diabetes display features of low grade inflammation years in advance of disease onset. Interleukin-6 is a proinflammatory cytokine. Therefore, A population-based study was undertaken to evaluate whether the promoter polymorphisms of interleukin 6 (IL-6; C-174G) genes predict the conversion from impaired glucose tolerance (IGT) to type 2 diabetes in the Kerala population. DNA was isolated from venous blood samples of T2DM patients (n=150) and normal healthy controls (n=150). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed after biochemical analysis. The genotypic and allelic frequency distributions were analyzed. The clinical/biochemical parameters of T2DM cases when compared to controls showed a significant difference. Significant association was observed with -174 G/C ($P<0.001$). Our data suggest that IL-6 gene polymorphisms play a prominent role in T2DM disease susceptibility in population from South Kerala.

I. INTRODUCTION

The incidence of diabetes mellitus in human population has reached epidemic proportions worldwide. India shelters the most number of people with diabetes mellitus worldwide with more than 62 million diabetic individuals currently diagnosed with the disease. Studies clearly estimate that ten years down the lane, one in every five diabetic patients will be an Indian.[1] Kerala, a state in India have a higher prevalence of diabetes since Kerala has the highest proportion of elderly in India. Studies clearly reported a high prevalence of Type 2 diabetes mellitus among adults above the age of 30 years in a representative population of South Kerala. [2] Although efforts to control hyperglycemia and associated symptoms are important, the major challenges in optimally managing the patient with DM are targeted at reducing or preventing complications, and improving life expectancy and quality of life.

Interleukin-6 is a pleiotropic cytokine belonging to the IL-6 family which include IL-11, oncostatin M, ciliaryneurotrophic factor, cardiotrophin-1, cardiotrophin like cytokine and leukemia inhibitory factor. It is a 26 kDa pleiotropic cytokine mainly produced by endothelial cells, macrophages, adipocytes and lymphocytes. It is involved in the regulation of the acute-phase reaction, immune responses, and hematopoiesis. Several studies have revealed the relation between IL-6 and pathogenesis of type 2 diabetes.[3] IL-6 increases postprandially, in parallel to glucose and insulin levels, in the interstitial fluid of subcutaneous adipose tissue. This increase suggests that IL-6 might modulate adipose glucose metabolism in the fed state. IL-6 mRNA expression and insulin resistance were found to have a significant correlation and increased plasma IL-6 levels with higher risk of T2DM, making it an appealing

candidate gene. Augmented levels of IL-6 are associated not only with T2DM but also with impaired glucose tolerance (IGT), indicating a potential role of this cytokine in its etiology.[4] One of the common polymorphisms in the IL-6 gene promoter (C-174G) was found to regulate transcription in response to inflammatory stimuli, such as lipopolysaccharides or IL-1. IL-6 promoter SNPs were considered as risk factors for T2DM development, as reported by other groups.[5] Studies on single nucleotide polymorphisms (SNPs) in the promoter region of IL-6 gene in different populations worldwide suggested its possible role in T2DM susceptibility. Therefore, we attempted to analyze the association of IL-6 promoter polymorphisms with T2DM patients from South Kerala population.

II. Materials & methods

Study area and population: A prospective study was conducted in Malappuram District of Kerala by conducting a medical camp at MES Medical College, 750 bedded tertiary care teaching hospitals at Perinthalmanna, Kerala. A total of 150 cases and 150 Control were enrolled into the study, informed consent was taken from all the subjects.

Inclusion criteria: Patients with DM with the Age between18 to 70 years were selected as the test population and standard population. The cases with Hyperglycaemia, history of diabetes or fasting blood glucose greater than or equal to 110 mg/dl were included as test population. People who are having normal blood glucose level were selected as control population.

Exclusion criteria: Woman who are pregnant, Individuals with Auto Immune Disease, Active malignancy, Chronic renal, hepatic, cardiac, gastrointestinal, skeletal or endocrine [except diabetes] diseases, Acute critical illness were excluded from the study. Sample were not considered in case of improper sample collection and the individuals suffering from STD's

The study was started after getting Institutional Ethical Committee approval. After obtaining an informed consent, patients were recruited to the study; their demographic data and Information like Body weight, Blood pressure, Details of other medication, food habit, height, and weight and waist circumference were collected by interviewing patients and also from the medical record. Blood samples were collected from patients and controls in the morning after 14–16 hours fasting. Plasma glucose (Fasting blood sugar and Post-prandial) (mg/dl) and lipid profile viz. total cholesterol (TC), triglycerides (TGL), high density lipoproteins (HDL) and serum creatinine (SCRT) were estimated using commercially available VITROS Chemistry Kits by UV-Vis spectrophotometer followed by low density lipoproteins (LDL) calculated by known formulae.

Genomic DNA was isolated from blood collected from the patients and controls by using GenElute Blood Genomic DNA Kit. DNA was amplified with primers specific for -174 G/C. The primers used were 5'-TGACTTCAGCTTTACTCTTTG-3', Reverse: 5'-CTGATTGGAAACCTTATTAAG-3' (Pishgam. Tehran, Iran). These specific PCR primers amplified a 198-bp fragment in which there is a specific restriction site to determine the different alleles of the rs1800795 SNP. PCR was carried out on an Eppendorf thermal cycler (Eppendorf, Hamburg, Germany) in a 15µl reaction mixture containing 100 ng of template DNA, buffer (100 mMTris, pH 9.0; 500 mMKCl; 15 mM MgCl2; 0.1% gelatin), 200µM deoxynucleotide triphosphate (dNTP), 10pmol of each primer (Integrated DNA Technologies, USA) and 1.5 units Taq DNA polymerase (BangloreGenei, India) under the following conditions: 95˚C for 5 min followed by 35 cycles of 95˚C for 30 s, 52˚C for 30 s, 72˚C for 45 s, and a final extension of 72˚C for 5 min. PCR products were then digested with 1 U of NlaIII restriction enzyme (Reaction Volume15 ML) (Fermentas, Lithuania, (FokIandNlaIII, Fermentas, USA)). After 4-h incubation at 37˚C, the enzyme cut PCR products into two fragments 213 and 135bp in length. The resulting products were visualized by 3.5% agarose gel electrophoresis.

In normal individual (also called as wild type) they have nucleotide G at 174 positions (for rs1800795 SNP) for mutant individual they have G getting converted to C at the 174 position for rs1800795 SNP). A person inherit different or same alleles for same gene from his or her parents, so after inheritance if an individual's IL6 gene at

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position 174 has GC genotype (ie G from one parent and C from other parent) it is classified as Heterozygous Mutant, GG genotype (ie G from one parent and G from other parent) it is classified as Homozygous Wild type, CC genotype (ie C from one parent and C from other parent) it is classified as Homozygous Mutant.

III. Results

The association between clinical/biochemical parameters of controls and T2DM patients showed a significant difference in FBS, PPBS, TC, TGL, HDL, LDL and VLDL (Table I). The IL-6 -174 G/C (rs1800795) polymorphisms were successfully genotyped in 150 T2DM cases and 150 healthy controls. Results of genotypic patterns by PCR-RFLP are shown in Fig. 1. The allele and genotype frequency distributions and carriage rates of polymorphism is shown in Table II,III and IV.

IL-6 -174 G/C (rs1800795) polymorphism showed significant genotypic and allelic associations (P<0.001) while, CC genotype was rare and GG was most prevalent in our population. GC genotype was present in 54.33 % controls and 20.67% T2DM cases (Table-3). The carriage rate of 'G' showed significant association (P<0.001) with T2DM when compared to controls but 'C' allele showed no association (Table-4).

Analysis of genotypic associations with biochemical parameters using multivariate logistic regression showed that subjects with GC showed significant association with all biochemical parameters except total cholesterol and CC showed significant association with FBS, PPBS and LDL (Table- 5).

Figure:

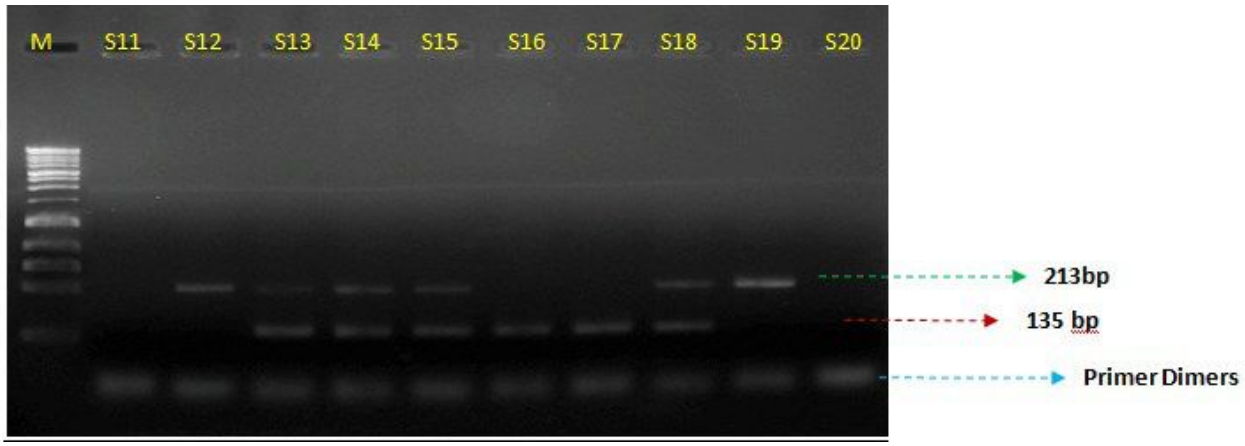

Fig. 1: photographs of PCR-RFLP products

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Tables:

Table I. Clinical characteristics of controls and T2DM cases

Clinical Characteristics	Controls (n=150)	Cases (n=150)
Age (yr)	45.58 ± 12.80	45.67 ± 11.20
Body weight	60.11±8.805	67.80± 16.41
Waist hip ratio (WHR)	0.94 ± 0.03	0.97 ± 0.66
Body mass index (BMI) kg/m2	24.1 ± 1.85	27.52 ± 8.15
Fasting blood glucose (FBS) mg/dl	85.28 ± 4.821	164.21 ± 12.76***
Post-prandial blood glucose (PPBS) mg/dl	139.65 ± 10.16	283.74 ± 36.16***
Total cholesterol (TC) mg/dl	183.36 ± 13.53	202.78 ±44.16***
Triglycerides (TGL) mg/dl	104.36 ± 16.91	95.83 ±11.2***
High density lipoproteins (HDL) mg/dl	47.36 ± 6.23	44.27 ± 10.13**
Low density lipoproteins (LDL) mg/dl	109.15 ± 13.00	136.54 ± 35.88***
Serum creatinine (SCRT) mg/dl	0.79 ± 0.386	0.68 ± 0.21

Table 2- Genotype frequencies

Genotypes	Controls Count (%) (n=150)	Cases Count (%) (n=150)
GG	107(71.33)	116(77.33)
GC	23(15.33)	31(20.67)
CC	20(13.34)	3(2.00)
P value	<0.001	

Table 3- Allele frequencies

	Controls Count (%) (n=150)	Cases Count (%) (n=150)
G	237(79.00)	263 (87.66)
C	63(21.00)	37(12.44)
P value	<0.001	

Table 4-Carriage rates

	Controls Count (%) (n=150)	Cases Count (%) (n=150)
G (+)	130(86.66)	147(98.00)
G (-)	20(13.34)	3(2.00)
P value	<0.001	
C (+)	43 928.67)	34 (22.77)
C (-)	107(71.33)	116(77.33)
P value	0.268	

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Table 5- Association of anthropometric/biochemical parameters with IL-6 -174 G/C genotypes

Genotypes ↓	Groups	AGE	BMI	WHR	FBS	PPBS	TC	TGL	HDL	LDL	SCRT
G/G	Controls (n=107)	45.03 ±8.43	24.06 ±1.354	0.0942 ±0.036	85.93 ±22.03	139.74 ±8.92	183.43 ±26.33	106.92 ±22.21	46.92 ±10.34	106.32 ±15.21	0.77 ±0.15
	Cases (n=116)	45.51 ±9.15	27.09 ±3.210	0.935 ±0.070	162.42 ±22.32	279.92 ±63.42	211.23 ±25.24	95.92 ±12.32	42.23 ±4.21	152.43 ±21.43	0.64 ±0.86
	P Value	0.067	0.132	0.347	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.26
G/C	Controls (n=23)	44.28 ±7.21	24.09 ±1.432	0.948 ±0.033	84.92 ±13.21	139.03 ±10.34	182.13 ±33.65	103.21 ±18.32	52.31 ±7.23	105.92 ±14.21	0.81 ±0.11
	Cases (n=31)	43.83 ±8.33	27.87 ±3.543	1.166 ±1.389	164.32 ±15.22	263.42 ±72.32	198.42 ±13.43	95.11 ±09.23	41.94 ±3.89	126.21 ±16.35	0.69 ±0.16
	P Value	0.643	0.081	0.591	<0.001	<0.001	0.731	0.043	<0.001	<0.001	1.02
C/C	Controls (n=20)	47.43 ±6.21	24.50 ±1.234	0.946 ±0.024	84.99 ±6.21	139.64 ±8.32	184.52 ±11.32	102.95 ±13.21	42.85 ±4.92	115.21 ±11.21	0.79 ±0.112
	Cases (n=3)	47.63 ±4.39	27.60 ±2.523	0.900 ±0.141	165.89 ±28.28	307.88 ±88.87	198.69 ±18.34	96.46 ±8.45	48.65 ±1.92	130.98 ±5.23	0.71 ±0.05
	P Value	.064	0.701	0.147	<0.001	<0.001	0.082	0.823	0.321	<0.001	0.423

IV. Discussion

In recent years, a large amount of evidence has accumulated indicating that insulin resistance and T2DM is closely related to a chronic, lowgrade inflammatory state of the body. This is the reason why circulating levels of IL-6 molecules remain elevated in people with T2D [6] and, furthermore, this value serves as an indirect measure for conditions of insulin resistance.[7] Earlier studies on the association of -174 G/C (rs1800795) SNPs with T2DM and insulin resistance (IR) and other diseases as well as with pre and post disease complications in almost all ethnic groups have demonstrated varied results among different populations. Moreover, till now only a few studies on -597 A/G (rs1800797) gene polymorphism have been reported and showed negative correlation with T2DM.[8]

A study on 1477 Koreans with normal glucose tolerance and 476 T2DM Korean patients to investigate the association of IL-6 gene polymorphisms with T2DM shows a result of Homozygosity for the rare G allele IL-6 -572C/G was associated with a higher risk of T2DM.[9] Serum IL-6 concentrations were associated with the IL-6 -572C/G genotype in control subjects. Also in the control group, subjects homozygous for the rare G allele showed significantly higher concentrations of hs-CRP than C/C and C/G carriers. The C-allele at the IL-6 -174 SNP was very rare and -597G/A and -1363G/T were monomorphic in this population. Hence the result demonstrate that the IL-6 -572G/G genotype is associated with higher serum IL-6 and hs-CRP concentrations and with increased risk for T2DM.

A population based study was to evaluate linkage between single-nucleotide polymorphisms known as risk factors and type 2 diabetes in an Indian population was failed to come across patients with mutation only in the IL-6 gene. But in another study, non diabetic subjects showed an association of IL-6-174 C/C genotype with higher insulin sensitivity.[10] Analyses of small cohorts of native Americans and Spanish Caucasians showed the 'G' allele of -174 G/C SNP to be associated with higher risk of T2DM[11], but this SNP was not linked with diabetes in the Finnish Diabetes Prevention Study (DPS).[12]

Genotyping in a north Indian study showed that -174 G/C SNP was significantly associated with T2DM.[13] Haplotype analysis showed that in combination, the -174C with -597G allele increased the risk and susceptibility of

developing T2DM in the north Indian population. The results showed that the allele 'G' was involved in disease prevalence and manifestation. 174 G/C (rs1800795) genotypic and allelic frequencies showed significant association. The results were in support of the metagenomic study in an Indian population26 which showed the prevalence of GG genotype. 'C' allele and CC genotype at -174 G/C SNP were found to be protective for diabetes as frequency of this genotype was more in control.

Hence Like other previous studies, our data suggest that IL-6 gene polymorphisms play a prominent role in determining susceptibility to T2DM. A negative association of Age, BMI, WHR and SCRT was observed in this study, but a significant interaction has been identified between FBS,PPBS, and LDL with all genotypes of -174 G/C (rs1800795) in gene polymorphisms. The prominent finding in respect to biochemical parameters was that LDL showed significant association with all genotypes of -174 G/C (rs1800795) gene polymorphisms while HDL showed association with only GG and GC genotype. TGL showed association with all genotypes except CC genotype of -174 G/C (rs1800795).

V. Conclusion

The clinical/biochemical parameters of T2DM cases when compared to controls showed a significant difference. G/C (rs1800795) polymorphism showed significant genotypic and allelic associations ($P<0.001$) and the carriage rate of 'G' showed significant association ($P<0.001$). Our data suggest that IL-6 gene polymorphisms play a prominent role in T2DM disease susceptibility in population from South kerala. And a significant interaction has been identified between FBS, PPBS, and LDL with all genotypes of -174 G/C (rs1800795) in gene polymorphisms.HDL and TGL showed association with all genotypes except CC genotype. Hence the present study provides a lead to the contribution of cytokine gene heterogeneity to the susceptibility and development of T2DM but it is essential to find out the degree of association in an individual population.

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