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Association of Genetic Polymorphism of Inflammatory Genes (IL-1β and IL-4) with Diabetes Type 2

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Abstract

It is known that genetic and environmental factors may influence susceptibility to type 2 diabetes (T2D). Pancreatic inflammation of T2D patients is associated with pathophysiology of diabetes type 2. In this study, we investigated role of polymorphism of IL-1β (rs16944) and IL4 gene and environmental factors in susceptibility to diabetes type 2.

190 diabetics and 210 healthy controls of central India were recruited. Anthropometric and lifestyle data were collected during sampling. Genetic polymorphism study of IL-1β gene was undertaken using PCR-RFLP meanwhile IL-4 polymorphism was investigated using PCR-VNTR (Variable number of tandem repeats) analysis. Genotype, allele frequencies and carriage rates of IL-1β and IL-4 polymorphisms were recorded.

Diabetic patients had higher levels of fasting plasma glucose (P<0.0001), HbA1c (P<0.0001) and Post prandial glucose (P<0.0001) compared to control subjects. Nominal difference observed for LDL-C (P=0.0462), triglyceride (P=0.0024). Diabetic patients had higher levels of weight of women (P=0.0024), Men (P=0.0157) and BMI of Women (P=0.0388), WHR in Women (P<0.0001) and WHR in Men (P=0.0147). Smoking habit data suggest odds ratio of smokers were 1.205 but statistically difference is not significant. Overall distribution of IL-β-511 genotypes was significantly different in Control as compared to diabetic patients. The frequencies of TT genotype (p =0.0047) and T allele ((P=0.0001) were significantly higher in patients than controls and associated with higher risk. An odds ratio of 1.781 of 'T' allele showed stronger association of overall "T' allele frequency in diabetes susceptibility. Overall IL-4 distribution of genotype was weakly but significantly different in case and control populations. Genotype distribution of IL-4 suggest association of B2B2 (p =0.0347) genotype with susceptibility to diabetes type 2 but allele frequency and carriage rate was not found significantly different. Our findings suggest association of IL-β-511 and weak association of IL-4 genotype as well as obesity with diabetes type 2.

Keywords: Diabetes type 2; Inflmmatory genes; IL-1; IL-4

Introduction

Diabetes is a metabolic disorder and now become global health problem having largest prevalence worldwide and it is world's sixth leading cause of death. T2D is mainly characterized as a state of hyperglycemia resulting from defects in insulin action and β-cell dysfunction. Etiology of diabetes is not very much Genetic susceptibility plays a crucial role in the etiology and manifestation of type II diabetes, with concordance in monozygotic twins approaching 100%. The complexity of type 2 diabetes is related to factors such as genetic heterogeneity, interactions between genes, and the modulating role played by the environment. Type 2 diabetes (T2D) is a complex metabolic disorder resulting from the interplay of both genetic and environmental factors like lifestyle and food habits. [1] Before twenty-first century it was generally suggested that inflammation and immune response can arise only in patients having diabetes type 1 but recently it has been seen that Inflammation is a key event closely associated with the pathophysiology of type 2 diabetes mellitus (T2DM).

Increasing clinical evidence is emerging that high-lights marked overlap between these two diabetic conditions. For example, immunological phenomena (e.g., anti-islet cell antibodies, elevated circulating cytokines and chemokines) classically associated with type 1 diabetes are present in many patients with type 2 diabetes [2,3], and obesity, which is associated with insulin resistance and type 2 diabetes. Cytokines plays a pathological role in diabetes, particularly interleukin-1 gene family of cytokine proteins.[4,5] Alleles of genes encoding immune/inflammatory mediators are associated with the disease. In present investigation we studied –511 in the promoter region polymorphism of IL-1β gene (rs16944) and VNTR polymorphism in intron 3 of IL4 gene with two major environmental factors obesity and smoking and its possible association in susceptibility to diabetes type 2. IL-1β cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. A European postdam study showed the possible role of interleukin 1β in susceptibility to diabetes type 2 and insulin resistance. [6] A promoter region polymorphism has been seen in this gene and has found to be strongly associated with many autoimmune as well as inflammatory disorders. Interleukin 4 is a potent regulator of inflammation as it regulates inflammatory response by increasing the expression of IL1RN and blocks TNF B and IFN Gamma. [7,8,9] IL-4 gene has a 70-bp variable number of tandem repeats (VNTR) polymorphism in intron 3 associated with IL-4 production.

Materials and Methods

Study Population

The study population consisted of 400 unrelated subjects comprising of 190 T2D patients and 210 ethnically matched controls of central Indian population were included in this study. Cases included consecutive patients who attended the Department of Medicine, Shyam Shah Medical College and Sanjay Gandhi Memorial Hospital, Rewa, Ayurveda Medical College, Rewa, Ranbaxy pathology Regional collection centre Rewa, District hospital Satna, Shahdol, Sidhi. Type 2 diabetes was diagnosed in accordance with World Health Organization (WHO Expert committee 2003) criteria. Pregnant women, children under age of 18 years and any patients with type 1 diabetes were excluded from the study.

Anthropometric and Biochemical Measurements

Anthropometry: Height and Weight were measured in light clothes and without shoes in standing position as per standard guidelines. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in standing position midway between iliac crest and lower costal margin and hip circumference was measured at its maximum waist to hip ratio (WHR) was calculated using waist and hip circumferences. Systolic and diastolic blood pressures were measured twice in the right arm in sitting position after resting for at least 5 minute using a standard sphygmomanometer and the average of the two reading was used.

Biochemical Analysis: Biochemical parameters related to type 2 diabetes were estimated for both cases and controls subjects. Measurement of Serum levels of Total cholesterol (TC), Triglycerides (TG), HbA1c, High density lipoprotein-cholesterol (HDL-C), Low density lipoprotein-cholesterol (LDL-C), Urea, Uric acid, C-reactive protein (CRP) and Creatinine were measured based on spectrophotometric method using automated clinical chemistry analyzer Cobas Integra 400 plus (Roche Diagnostics, Mannheim, Germany). The protocol and principle of biochemical analysis has already been discussed in review section.

Blood Collection and Plasma/Serum Separation

Venous blood samples were obtained from the subjects after 12 hours of overnight fasting in vacutainers with and without appropriate anti-coagulants. Immediately, plasma and serum from the respective vacutainers were separated by centrifuging the tubes at 1000 rpm for 10 min. at 4˚C.

DNA Isolation and Quantification

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers. [10] The isolated genomic DNAs were then tested for purity by measuring their absorbance values at 230 nm, 260 nm, 280 nm and 300 nm using a UV visible spectrophotometer (Systronic, India). A DNA preparation was considered to be good if it had A 260 nm / A 280 nm ratio as approximately 1.8 and A 300 nm was 0.1 or lesser. Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appears as single band. A horizontal agarose slab gel electrophoresis apparatus (Bangalore Genei, Bangaore, India) was used.

Detection of Interlukin-1 Beta (IL1-β) Single Nucleotide Polymorphism via PCR-RFLP

The nucleotide position -511 in the promoter region of this gene has a single nucleotide polymorphism that results in change of nucleotide from cytosine (C) to thymine (T). The oligonucleotide sequence (primers) were designed to create a recognition site for the restriction enzyme AvaI in allele 1 (C at -511 position) but no restriction site in allele 2 (T at -511 position) of IL-1β gene.

Primer Sequences

The oligonucleotides sequences (primers) used were those described before. [11]

IL1-β forward primer - 5' TGGCATTGATCTGGTTCATC 3'
IL1-β reverse primer - 5' GTTTAGGAATCTTCCCACTT 3'

PCR Mix

For each DNA sample 25 μl of PCR reaction mixture was prepared containing 5 μl template DNA (final concentration 100-200 ng/ μl), 2.5 μl of 10X Taq polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; Genetix Biotech Asia Pvt. Ltd., India), 1 μl of 10 mM dNTPs (Banglore Genei, Bangalore, India), 1 μl of 25 pmol/μl of forward and reverse primers, 0.2 μl of 5U/ μl of Taq DNA polymerase (final concentration 1U; Genetix Biotech Asia Pvt. Ltd.,India) and sterile water to set up the volume of reaction mixture to 25 μl.

Thermal Profile

Thermal profile used for the amplification of desired segment of gene was as follows: Initial denaturation at 95˚C for 2 min and 35 cycles of denaturation at 95˚C for 1 min, annealing at 54˚C for 1 min and extension at 74˚C for 1 min, followed by final extension at 74˚C for 10 min. [11] PCR products were separated on 2% agarose gel (2% w/v, Sigma) using a 100 bp molecular weight (MW) marker to confirm the PCR product size of 304 bp.

Restriction Digestion by *Ava*I

The C to T transition in promoter region of IL1- β gene when amplified by PCR was than incubated with AvaI restriction enzyme (New England Biolabs,USA). Digestion of the amplified 304 bp PCR product gave two fragments in PAGE of 190 bp and 114 bp respectively if the product was excisable by *Ava* I. Depending on digestion pattern, individuals were scored as genotype CC when homozygous for presence of *Ava* I site, genotype TT when homozygous for absence of *Ava* I site and genotype CT in case of heterozygosity (Figure 1).

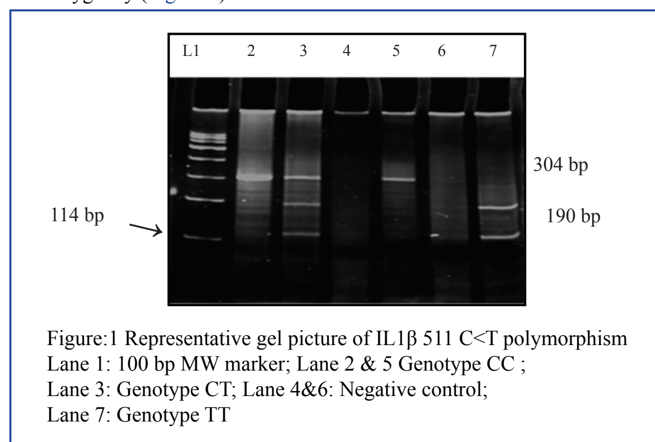

Figure:1 Representative gel picture of IL1β 511 C<T polymorphism
Lane 1: 100 bp MW marker; Lane 2 & 5 Genotype CC ;
Lane 3: Genotype CT; Lane 4&6: Negative control;
Lane 7: Genotype TT

Genotyping

3 possible genotypes were expected depending upon the PCR pattern (Figure 2). They are as follows: Genotype B1B1 (2 VNTR of 70 bp) 183bp genotype B2B2 (253 bp), Genotype B1B2 (183bp and 253 bp).

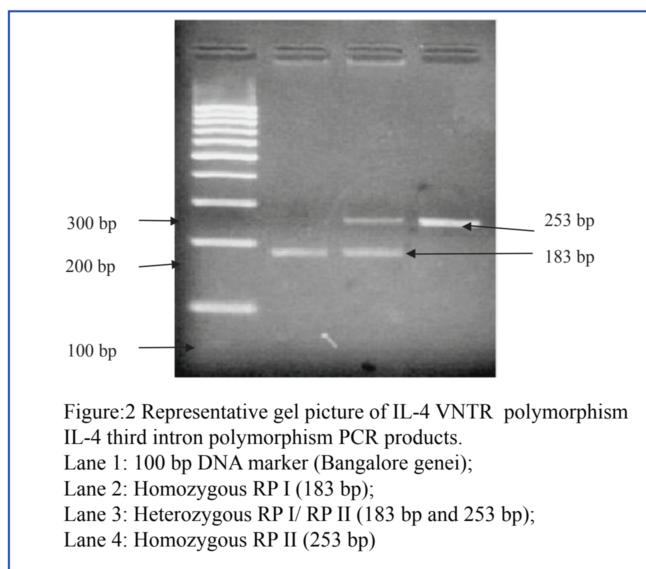

Figure:2 Representative gel picture of IL-4 VNTR polymorphism
IL-4 third intron polymorphism PCR products.
Lane 1: 100 bp DNA marker (Bangalore genei);
Lane 2: Homozygous RP I (183 bp);
Lane 3: Heterozygous RP I/ RP II (183 bp and 253 bp);
Lane 4: Homozygous RP II (253 bp)

Detection of Interleukin 4 (IL4) 70 bp VNTR DNA Polymorphism

IL4 gene of 10 kbp composes of four exons and three introns. [12] In the present study, we investigate the polymorphisms in the third intron region of the IL-4 gene. The polymorphisms consist of a variable number of tandem repeats (VNTRs) of a 70 bp repeating sequence.

Primers

The oligonucleotides sequences (primers) used were those described before. [12]
Forward `5-AGGCTGAAAGGGGGAAAGC- 3`
Reverse 5`- CTGTTCACCTCAACTGCTCC- 3`

Master Mix Preparation

For each DNA sample 25 µl of PCR reaction mixture was prepared containing 5-6 µl template DNA (final concentration 100-200 ng/ µl), 2.5 µl of 10X Taq polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; Genetix Biotech Asia Pvt. Ltd., India), 1 µl of 10 mM dNTPs (Banglore Genei, Bangalore, India), 1 µl of 25 pmol/µl of forward and reverse primers specific for IL4 gene, 0.2 µl of 5U/ µl of Taq DNA polymerase (final concentration 1U; Genetix Biotech Asia Pvt. Ltd., India) and sterile water to set up the volume of reaction mixture to 25 µl.

Thermal Profile

The cycling conditions were 95°C for 10 minutes, followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and final extension at 72°C for 8 minutes. PCR products were separated on 2% agarose gel (2% w/v, Ambrasco, Fermantas) using a 100 bp molecular weight (MW) marker to confirm different PCR product sizes indicating presence of different alleles of VNTR.

Anthropometric Results with Life Style Factors

The descriptive data and comparison of anthropometric parameters of diabetic patients versus controls are presented in Table 1. As expected the diabetic patients had markedly higher levels of weight of women (P=0.0024), Men (P=0.0157) and BMI of Women (P=0.0388), Waist circumference in women (P<0.0001), WHR in Women (P<0.0001) and WHR in Men (P=0.0147). Other results were not significantly different between case and control group and are tabulated in table 3(A) (See Table 1).

Smoking habit data were also collected during questionnaire organized during sample collection. Bidi and cigarette smokers were included as smoking population. The data indicates the percentage of smokers was not very much different between both in case and control population (25.79% Vs 22.38%) and not any significant difference was seen. Odds ratio of smokers were 1.205 which indicates that possibly smoking could be a risk factor associated with diabetes type 2 pathophysiology of diabetes type 2 although the mode of effects are not very much clear (Table 2).

Biochemical and Clinical Findings

Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t-test and p-value obtained suggests the level of significant changes here. The descriptive data and comparison of biochemical parameters of diabetic patients versus controls are presented in Table 3(B). As expected the diabetic patients had markedly higher levels of fasting plasma glucose

(P<0.0001) and HbA1c (P<0.0001) and Post prandial glucose (P<0.0001) compared to that of control subject. Nominal difference was also observed for LDL-C (P=0.0462), triglyceride (P=0.0024), systolic blood pressure (P=0.0447). creatinine value, blood urea level, HDL-C level and diastolic pressure was not significantly different between two groups and all the clinical test results are tabulated in Table 3.

TABLE No- 1 Comparison of anthropometric parameters

Characteristics	Cases	Controls	P-value
n(Men/Women)	190(126/64)	210(114/96)	
Age(years)	52.5±12.5	53.0±14.2	0.7100
Height(m)	160.50±13.40	162.2± 12.000	0.1815
Weight (Kg)			
Women	62.5 ±5.70	60 ± 4.50	0.0024 **
Men	68±5.60	66.0±7.1	0.0157*
BMI (kg/m^2)			
Women	26.4±3.1	25.1 ± 4.3	0.0388*
Men	24.6±4.7	24.1± 5.1	0.4301
Waist circumference (cm)			
Women	92.5±6.2	84.5±6.7	P<0.0001***
Men	90.0±7.0	89.0±6.0	0.2383
Hip (cm)			
Women	95.0±5.0	96.5±6.0	0.178
Men	91.0±4.0	90.5±5.5	0.4183
WHR			
Women	0.97±0.05	0.88±0.08	P<0.0001***
Men	0.99±0.05	1.00±0.03	0.0147*

* denotes level of significant change between case and control

Table 2 Life style factors

Life style factors	CASE N= 190		CONTROL N=210		Chi square value χ^2 (P Value)	ODDS RATIO AND CI
	n	%	n	%		
Smoking Habits	49	25.79	47	22.38	0.6354 (0.4254)	1.205 (0.7613 - 1.908)

* denotes level of significant change between case and control

Table-3 Comparison of Biochemical and clinical findings of diabetic patients and controls

Characteristics	Cases	Controls	P-value
FPG(mg/dL)	143.3± 17.6	92.44±7.5	P<0.0001***
Post-Prandial Glucose (mg/Dl)	211.7±44.7	108.5±12.1	P<0.0001***
HbA1C(%)	6.9±0.8	5.3±0.6	P<0.0001***
HDL-C(mmol/L)	112.2±14.8	109.8±11.6	0.0705
LDL-C (mg/dL)	42.1±4.3	41.3±3.7	0.0462*
TG(mg/dL)	131.1±13.2	126.9±14.2	0.0024**
Systolic BP (mmHg)	130.20±8.1	128.8±5.7	0.0447*
Diastolic BP (mmHg)	87.1±5.8	86.5±6.0	0.3109
Blood Urea(mg/dL)	9.1±1.6	8.8±1.8	0.0801
Creatinine(mg/dL)	1.08±0.14	1.06±0.10	0.0986

(* denotes the level of significant change between case and control)

Detection of Genetic Polymorphism in IL-1β

The distribution of the polymorphisms of IL-1β (rs16944) was consistent with Hardy- Weinberg equilibrium (HWE) in case and healthy control groups. Overall distribution of IL-1β-511 genotypes was significantly different in Healthy Control group as compared to disease group. HC group showed a significant increase in '*CC*' genotype as compared to Patients of diabetes type 2 (18.10% Vs 6.84%). Similarly, '*TT*' genotype was present in significantly lower frequency in HC as compared to Diabetes type 2 patients group (51.58% vs. 37.14%), Overall allele '*C*' was found in significantly lower frequency in disease group as compared to HC group whereas allele '*T*' was present in significantly high frequency in the disease group (χ^2 =14.59, P value= 0.0001). An odds ratio of 1.781 of '*T*' allele showed stronger association of overall 'T' allele frequency in diabetes susceptibility. Carriage rate of allele '*C*' was significantly higher in HC group whereas carriage rate of allele 'T' was high in disease group (χ^2 =5.096, P value 0.0240). The frequencies of TT genotype and T allele were significantly higher in patients than controls and associated with high risk (p =0.0047; OR= 1.803, 95% CI 1.209- 2.687 and p=0.0001; OR=1.781, 95% CI 1.323 -2.398 respectively). On the other hand, the CC genotype and C allele had low risk (p=0.0008; OR=0.3324, 95% CI 0.1711- 0.6458 and p=0.0001; OR=0.5615, , 95% CI 0.4170- 0.7561 respectively). (Table 4)

Table No.4 Fisher Exact Test values of IL-1β 511 C/T polymorphism

IL-1β GENOTYPE	CASE N= 190		CONTROL N=210		P Value	ODDS RATIO (95% CI)
	n	%	n	%		
CC	13.0	06.84	38	18.10	0.0008***	0.3324 (0.1711- 0.6458)
CT	79	41.57	94	44.76	0.5453	0.8783 (0.5907- 1.306)
TT	98	51.58	78.0	37.14	0.0047**	1.803 (1.209- 2.687)
ALLELES						
C	105	27.63	170	40.47	0.0001***	0.5615 (0.4170- 0.7561)
T	275	72.37	250	59.53		1.781 (1.323 -2.398)
CARRIGE						
C	92	51.11	132	62.85	0.0259*	0.6773 (0.4825- 0.9506)
T	177	93.16	172	81.90		1.476 (1.052 - 2.072)

(* denotes the level of significant change between case and control)

Detection of Genetic Polymorphism In IL-4

The genotypes of IL-4 were under accordance of HWE. The distribution of genotype distribution was weakly but significantly different in case and control populations but the carriage rate and allele frequency were not different significantly. In present study we found genotype B1/B1 was slightly higher in diabetic case population as compared to control but difference was nominal and statistically nonsignificant (8.24% Vs 5.24%). The genotype B2/B2 was found in significantly higher frequency in case population as compared to healthy controls (60.00% vs. 49.04%). Overall allele B1 were present in slightly higher percentage in control as compare to diabetic patients (28.10 in healthy control Vs 24.21% in Case) meanwhile the frequency of B2 were slightly higher in diabetic patients but difference were not significantly different ($\chi 2$=1.555, P value=0.2124). An odds ratio of 1.231 of B2 allele carriage showed little or no effect of B2 allele carriage in diabetes susceptibility. On the basis of Genotype distribution we found association of B2B2 (p =0.0347; OR= 1.558, 95% CI 1.048 - 2.317) genotype with susceptibility to diabetes type 2 despite the allele frequency and carriage rate analysis was not found significantly different meanwhile B1B2 Genotype was protective (p =0.0041; OR= 0.5317, 95% CI 0.3470 - 0.8149). (Table 5)

Table no. 5 Fisher Exact Test values of IL4 VNTR polymorphism

IL-4 GENOTYPE	CASE N= 190		CONTROL N=210		P Value	ODDS RATIO (95% CI)
	N	%	N	%		
B1B1	16	08.42	11	5.24	0.2343	1.664 (0.7517 - 3.681)
B1B2	60	31.58	96	45.71	0.0041**	0.5317 (0.3470 - 0.8149)
B2B2	114	60.00	103	49.04	0.0347*	1.558 (1.048 - 2.317)
ALLELES						
B1	92	24.21	118	28.10%	0.2278	0.8176 (0.5955 - 1.122)
B2	288	75.79	302	71.90%		1.223 (0.8910 - 1.679)
CARRIGE RATE						
B1	76	40.0	107	50.95	0.2767	0.8123 (0.5681 - 1.162)
B2	174	91.58	199	94.76		1.231 (0.8609 - 1.760)

(* denotes the level of significant association between case and control)
N – Number of individuals in study group %- Genotype allele frequency and carriage rate expressed in percentage
%- Genotype allele frequency and carriage rate expressed in percentage

Discussion

Type 2 Diabetes Mellitus is a disease that is increasing rapidly in a global environment of obesity and sedentary lifestyles. Subclinical, low-grade systemic inflammation has been observed in patients with diabetes type 2 and in those at increased risk of the disease. This may be more than an epiphenomenon. Alleles of genes encoding immune/inflammatory mediators are associated with the disease, and the two major environmental factors that contribute to the risk of type 2 diabetes—diet and physical activity—have a direct impact on levels of systemic immune mediators. [3] IL-1β cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. A European postdam study showed the possible role of interleukin 1β in susceptibility to diabetes type 2 and insulin resistance. [6] Recent studies from animals, in-vitro cultures and clinical trials provide evidence that support a causative role for IL-1β as the primary antagonist in the loss of beta-cell mass in type 2 diabetes. In vitro, IL-1beta-mediated autoinflammatory process results in beta-cell death. The auto-inflammation is driven by glucose, free fatty acids, leptin, and IL-1β itself. Caspase-1 is required for IL-1beta activity and the release of free fatty acids from the adipocyte. [13] It has been proved that the mechanisms involved in inflammation and specifically the IL-1β signals that lead to the progression of insulin resistance and diabetes. [14] Genetic polymorphism in IL-1 β 511 C/T has been widely studied before and was found to be significantly associated with many autoimmune disorders and type of many cancers. The promoter region 511-C/T polymorphism may modulate the expression of interleukin 1 β and C allele and has been found to be less active as compare to T allele and in case of T carrier there is an increase in IL1β production which can be associated with potent inflammatory response. [15]

In our study we found a very strong and significant association of IL1 β C<511>T polymorphism with susceptibility to diabetes type 2. The frequencies of TT genotype and T allele were significantly higher in patients than controls and associated with higher risk of diabetes (p =0.0047; OR= 1.803, 95% CI 1.209- 2.687 and p=0.0001; OR=1.781, 95% CI 1.323 -2.398 respectively). On the other hand, the CC genotype and C allele had low risk (p=0.0008; OR=0.3324, 95% CI 0.1711- 0.6458 and p=0.0001; OR=0.5615, 95% CI 0.4170- 0.7561 respectively). Our study strongly supports the previous study done in north Indian population.[16] IL1β has been also found to be associated with diabetic nephropathy in korean population.[17] Interleukin-1β polymorphism (T allele) has also been found to be associated with diabetic nephropathy in the Northern Ireland population. [18] Another study done in Malaysian population showed lack of association with diabetes type 2. [19] This variation may due to different population origin or different environmental effects. A European postdam study showed the possible role of interleukin 1β in susceptibility to diabetes type 2 and insulin resistance. [6]

Interleukin 4 is a potent regulator of inflammation as it regulates inflammatory response by increasing the expression of IL1RN and blocks TNF B and IFN Gamma. In patients of diabetes type 2 a chronic inflammation has been seen and probably this inflammation may cause insulin resistance and apoptosis in pancreatic beta cells. IL-4 gene has a 70-bp variable number of tandem repeats (VNTR) polymorphism in intron 3 associated with IL-4 production.

In present investigation we studied the association of genetic polymorphism of interleukin 4 with diabetes type 2. The genotype B2/B2 was found in significantly higher frequency in case population as compared to healthy controls (60.00% vs. 49.04%). Heterozygous was significantly higher in control (45.71% vs. 31.58%). On the basis of Genotype distribution we found association of B2B2 (p =0.0347; OR= 1.558, 95% CI 1.048 - 2.317) genotype with susceptibility to diabetes type 2 despite the allele frequency and carriage rate analysis was not found significantly different meanwhile B1B2 Genotype was protective (p =0.0041; OR= 0.5317, 95% CI 0.3470 - 0.8149). A previous study in north Indian also showed that IL-4 B2B2 genotype was higher in patients of diabetes type 2 with odds ratio 2.30. [20] Our study favors this north Indian study and shows a significant association of heterozygous genotype with type 2 diabetes. Previously IL-4 VNTR polymorphism has been widely studied and found inconsistently associated with diabetes type 1, this is because of its role in immunoregulation and regulation of inflammation. Previously one polymorphism in interleukin 4 at 590 positions has been found to be associated with diabetes type 2 and nephropathy in south-east Iranian Patients. [21] A probable reason for the similarity between our results and those of Bid and colleagues could be that north Indian populations are similarity in race and genetics from our studied population. While Iranian population may have a different race but those both studies shows a significant association with diabetes type 2 with polymorphism in IL-4 and our results are consistent with those studies. It was proposed that an increased responsiveness of the RP1 allele to transcriptional activation might lead to over expression of IL-4 thus increased expression may cause improvement in IL-1Ra production and inflammation could be reduced. [22,23] A large sample size is required to study the effect of IL-4 with diabetes type 2 because present investigation was in a small sample size.

BMI indexing is a tool used for documentation of obesity. In our present investigation we found that BMI was significantly higher in females. BMI of diabetic females were as compared to healthy females (P=0.0388). Weight Height Ratio (WHR) was also shown to be higher in male and female both. Sedentary lifestyle is strong factor behind the surprising rise in the prevalence of both obesity and diabetes. [24] In the past decade, we have witnessed an epidemic of both diabetes type 2 and obesity. The recent increase in the prevalence of obesity is closely paralleled by the increase in the prevalence of diabetes. Indeed, this new unprecedented phenomenon has been referred to as "diabesity." There is a clear strong relationship between obesity and the risk for diabetes. [25] In India our data also suggests that obesity and higher BMI can be an important factor which can affect the susceptibility to diabetes type 2. It has been established before that with lifestyle modification (weight loss, regular moderate physical activity), diabetes can be delayed or prevented. [26-28]

Smoking is an established modifiable risk factor which is associated with many diseases such as CVD [29,30] and cancer. To some extent, the effects in physical conditions of smoking and diabetes are similar, which brings question if there is any association between smoking and diabetes. Many studies evidenced that chronic smokers have a higher risk for insulin resistance, and to develop diabetes type 2 mellitus (DM2). [31-34] Our result shows that percentage of smokers in case and control is not more different and there is lack of statistically significant association but an odds ratio of 1.205 shows a little higher risk of diabetes type 2 in smokers as compared to nonsmokers. Many other studies also show that current smokers have a 1.2- to 2.6-times higher risk of diabetes type 2 than nonsmokers. [35-39] Our results are consistent with this finding obtained by this meta analysis but risk of diabetes is lower with smoking in our result as compare to results of this meta analysis.

In present investigation we had taken small sample size but this is the first molecular level study in vindhyan region (Central India) population and we recommend a Genome wide association study in this region to find out the other genes associated with diabetes type 2. The replication of this study with larger sample size will be expected in future. Despite those genes many other genes can have strong effect in diabetes pathophysiology so we can use genome wide association study to discover some novel genes which have strong association with diabetes type 2 susceptibility. If consistent research would be done to combat diabetes we will find permanent solution and would have better medicines against type 2 diabetes.

Conclusion

On the basis of our findings we can conclude that obesity is associated with diabetes susceptibility only in female subjects meanwhile polymorphism in IL1β is strongly but IL-4 VNTR polymorphism is weakly associated with diabetes risk. Smoking habit data suggests lack of association of smoking with diabetes risk.

Reference

1. McCarthy MI, Froguel P (2002) Genetic approaches to the molecular understanding of type 2 diabetes. Am J Physiol Endocrinol Metab 283: E217-225
2. Pietropaolo M, Barinas-Mitchell E, Pietropaolo SL, Kuller LH, Trucco M (2000) Evidence of islet cell autoimmunity in elderly patients with type 2 diabetes. Diabetes 49: 32-38.

3. Kolb H, Mandrup-Poulsen T (2005) An immune origin of type 2 diabetes? Diabetologia 48: 1038-1050.
4. Tuttle HA, Davis-Gorman G, Goldman S, Copeland JG, McDonagh PF (2004) Proinflammatory cytokines are increased in type 2 diabetic women with cardiovascular disease. J Diabetes Complications 18: 343-351.
5. Mandrup-Poulsen T, Zumsteg U, Reimers J, Pociot F, Mørch L, et al. (1993) Involvement of interleukin 1 and interleukin 1 antagonist in ancreatic beta-cell destruction in insulin-dependent diabetes mellitus. Cytokine 5: 185-191.
6. Spranger J, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, et al. (2003) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 52: 812-817.
7. Mijatovic T, Kruys V, Caput D, Defrance P, Huez G (1997) Interleukin-4 and -13 inhibit tumor necrosis factor-alpha mRNA translational activation in lipopolysaccharide-induced mouse macrophages. J Biol Chem 272: 14394-14398.
8. McBride WH, Economou JS, Nayersina R, Comora S, Essner R (1990) Influences of interleukins 2 and 4 on tumor necrosis factor production by murine mononuclear phagocytes. Cancer Res 50: 2949-2952.
9. Opal SM, DePalo VA (2000) Anti-inflammatory cytokines. Chest 117: 1162-1172.
10. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215.
11. Chaudhary AG (2008) IL-1B Gene Polymorphism and Susceptinility to Rheumatoid Arthritis in Ethnic Saudi Patients. World Applied Sciences J 5: 449-454.
12. Arai N, Nomura D, Villaret D, DeWaal Malefijt R, Seiki M, et al. (1989) complete nucleaotide sequence of the chromosomal gene for IL-4 and its expression. J Immunol 142: 274-282.
13. Dinarello CA, Donath MY, Mandrup-Poulsen T (2010) Role of IL-1beta in type 2 diabetes Curr Opin Endocrinol Diabetes Obes 17: 314-321.
14. Maedler K, Dharmadhikari G, Schumann DM, Størling J (2009) Interleukin-1 beta targeted therapy for type 2 diabetes. Expert Opin Biol Ther 9: 1177-1188.
15. Furuta T, El-Omar EM, Xiao F, Shirai N, Takashima M, et al. (2002) Interleukin 1beta polymorphisms increase risk of hypochlorhydria and atopic gastritis and reduce risk of duodenal ulcer recurrence in japan. Gastroenterology 123: 92-105.
16. Achyut BR, Srivastava A, Bhattacharya S, Mittal B (2007) Genetic association of interleukin-1beta (-511C/T) and interleukin-1 receptor antagonist (86 bp repeat) polymorphisms with Type 2 diabetes mellitus in North Indians. Clin Chim Acta 377: 163-169.
17. Lee SH, Ihm CG, Sohn SD, Lee TW, Kim MJ, et al. (2004) Polymorphisms in Interleukin-1β and Interleukin-1 Receptor Antagonist Genes Are Associated with Kidney Failure in Korean Patients with Type 2 Diabetes mellitus Am J Nephrol 24: 410-414.
18. Loughrey BV, Maxwell AP, Fogarty DG, Middleton D, Harron JC, et al. (1998) An interleukin 1B allele, which correlates with a high secretor phenotype, is associated with diabetic nephropathy. Cytokine 10: 984-988.
19. Vasudevan R, Patimah I, Johnson S, Norashikin S (2008) C-511T polymorphism of Interlukin -1beta Gene is not Associated in Type 2 Diabetes Mellitus-A study in Malaysian population. J Medical Sci 8: 216-221.
20. Bid HK, Konwar R, Agrawal CG, Banerjee M (2008) Association of IL-4 and IL-1RN (receptor antagonist) gene variants and the risk of type 2 diabetes mellitus: a study in the north Indian population. Indian J Med Sci 62: 259-266.
21. Arababadi MK, Pourfathollah AA, Daneshmandi S (2009) Evaluation of relation between IL-4 and IFN-g p Olsson T. (1995) Cytokine-producing cells in experimental autoimmune encephalomyelitis and multiple sclerosis. Neurology 45: S11-S15.
22. Vandenbroeck K, Martino G, Marrosu M, Consiglio A, Zaffaroni M, et al. (1997) Occurrence and clinical relevance of an interleukin-4 gene polymorphism in patients with multiple sclerosis. J Neuroimmunol 76: 189-192.
23. Olsson T (1995) Cytokine-producing cells in experimental autoimmune encephalomyelitis and multiple sclerosis. Neurology 45: S11-S15.
24. Blair SN, Church TS (2003) The Importance of Physical Activity and Cardiorespiratory Fitness for Patients With Type 2 Diabetes Diabetes Spectrum 16: 236-240.
25. Caprio S (2003) Obesity and Type 2 Diabetes: The Twin Epidemic. Diabetes Spectrum 16: 230.
26. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, et al. (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 346: 393-403.
27. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, et al. (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344: 1343-1350.
28. Pan XR, Li GW, Hu YH, Wang JX, Yang WY, et al. (1997) Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care 20: 537-544.
29. Burke AP, Farb A, Malcom GT, Liang Y, Smialek J, et al. (1997) Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. N Engl J Med 336: 1276-1282.
30. Greenland P, Knoll MD, Stamler J, Neaton JD, Dyer AR, et al. (2003) Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA 290: 891-897.
31. Carlsson S, Midthjell K, Grill V, Nord-Trøndelag study (2004) Smoking is associated with an increased risk of type 2 diabetes but a decreased risk of autoimmune diabetes in adults: an 11-year follow-up of incidence of diabetes in the Nord-Trondelag study. Diabetologia 47: 1953-1956.

32. Willi C, Bodenmann P, Ghali WA, Faris PD, Cornuz J (2007) Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 298: 2654-2664.
33. Eliasson B (2003) Cigarette smoking and diabetes. Prog Cardiovasc Dis 45: 405-413.
34. Facchini FS, Hollenbeck CB, Jeppesen J, Chen YD, Reaven GM (1992) Insulin resistance and cigarette smoking. Lancet 339: 1128-1130.
35. Rimm EB, Manson JE, Stampfer MJ, Colditz GA, Willett WC, et al. (1993) Cigarette smoking and the risk of diabetes in women. Am J Public Health 83: 211-214.
36. Manson JE, Ajani UA, Liu S, Nathan DM, Hennekens CH (2000) A prospective study of cigarette smoking and the incidence of diabetes mellitus among US male physicians. Am J Med 109: 538-542.
37. Persson PG, Carlsson S, Svanstrom L, Ostenson CG, Efendic S, et al. (2000) Cigarette smoking, oral moist snuff use and glucose intolerance. J Intern Med 248: 103-110.
38. Wannamethee SG, Shaper AG, Perry IJ, British Regional Heart Study (2001) Smoking as a modifiable risk factor for type 2 diabetes in middle-aged men. Diabetes Care 24: 1590-1595.
39. Will JC, Galuska DA, Ford ES, Mokdad A, Calle EE (2001) Cigarette smoking and diabetes mellitus. Evidence of a positive association from a large prospective cohort study. Int J Epidemiol 30: 540-546.

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