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Original Research Article

ASSOCIATION OF SDF-1β GENE SINGLE NUCLEOTIDE POLYMORPHISM IN TYPE 2 DIABETES MELLITUS PATIENTS IN NORTH INDIAN POPULATION

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ABSTRACT

Introduction: T2D affects over 340 million people worldwide. T2D affects low – and middle – income populations accounting for more than 80% of the deaths due to diabetes. The genetic variants associated with T2D are complicated by lifestyle and environmental factors that play a major role in disease onset and progression. This research work is an attempt to contribute in the search for genes responsible for complex forms of T2DM.

Objective of the Study: Our study explores the association of SDF- 1β between genetic variants and T2DM in North Indian Population.

Materials and Methods: This study was retrospective, analytical case control study & was carried out in the Central Research lab of Subharti Medical College. Meerut. Peripheral blood was collected from 150 unrelated T2DM patients attending Out – patient and In-patient departments of medicine C.S.S.H Meerut. India for diabetes & 150 healthy controls. Whole venous blood was collected & used for DNA extraction. DNA was further amplified by the technique of polymerase chain reaction (PCR). Two sets of primers were used in PCR i-e forward and reverse primer. These fragments were further subjected to the digestion by restriction endonuclease enzyme Msp1 and were subjected to agarose gel electrophoresis.

Results: The odd ratio of SDF -1β genotypes and alleles were calculated in T2DM and compare them with control subjects. The frequency of SDF -1 ß G / G homozygous wild in T2DM showed a significantly high relative risk, when compared with healthy controls. Relative risk associated with this genotype was 4.500. p value was calculated to be < 0.0001.

Conlusion: On the basis of our study we can conclude that The "GG" genotype of SDF-1β gene is confined to be the risk allele and is associated with T2DM patients in North Indian population. Although this study focused on a relatively small number of individuals, its finding contribute to the growing evidence of the presence and effects of genetic variant in the understudied North Indian population.

KEY WORDS: T2DM, SDF- 1β gene, Genotypes, Alleles.

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INTRODUCTION

Diabetes Mellitus (DM), the most prevalent metabolic disease is a multifactorial disorder which is influenced by both environmental and

genetic factors. It has been estimated that approx 10% of the population in Japan and Western countries is victim of these disease [1]. Increased prevalence of DM has been observed in Asian countries especially India [2]. In order to study this trend, a national survey was conducted in India and it was estimated that approximately 12% of population settled in the urban areas was suffering from the disease [3,4]. World Health Organization published a data in 2000, according to which at least 171 million people Worldwide suffered from diabetes or 2.8% of the population was diabetic. Unfortunately the incidence of diabetes is increasing even more rapidly. According to this increasing trend it is expected the total number of people worldwide with diabetes rises from 285 million in 2010 to 439 million in 2030 corresponding to a predicted increase in prevalence from 6.4% in 2010 to 7.7% in 2030 [5]. Two basic abnormalities have been observed in the development of T2DM which include impaired synthesis and release of insulin secretion by β – cells of pancreas and decreased insulin sensitivity [6].

At present treatment of T2DM is restricted to medical therapies that ameliorate the condition and do not aim to restore normal glucose metabolism and the patients are open to the risk of life threatening hurdles. Understanding the origin of the genetic traits of T2DM may possibly show the way to the identification of new therapeutic goal which represents one of the most promising approach for enduring treatment achievement.

Underlying molecular events responsible for this disease have been studied thoroughly for the last three to four decades but unfortunately in spite of a lot of hard work, the basic fundamental events are still to be explored completely [7-9]. Human chemokines are a large family of molecules characterized by structural homologies based on conserved cysteine residues, as well as binding capacities to particular G- protein – coupled receptors [10,11]. Chemokines are key mediators in the pathogenesis of inflammatory, auto- immune, vascular and neoplastic disorders. Stromal cell- derived factors 1 alpha and beta (SDF1a and SDF1h) are small cytokines belonging to the intercrine CXC subfamily originally isolated from a murine marrow stromal cell line. Currently no comprehensive information for genetic association studies of T2DM has been reported in North Indian population. Therefore our study will add a layer in the field of genetics as we aimed to explore the association of SDF-β between genetic variants & T2DM in North Indian population in our study.

MATERIALS AND METHODS

The study was carried out in the Central Research lab of Subharti Medical College. Meerut. This study was retrospective, analytical case control study. The study protocol was approved by the Institutional ethical committee. Prior to sample collection, written informed consent was taken from all the subjects, included in the study explaining the nature of the project. Peripheral blood was collected from 150 unrelated T2DM patients attending Out- and in- patient department of medicine C.S.S.Hospital, Meerut, India for diabetes & 150 healthy controls. Both the groups were of same ethnic origin.

Inclusion Criteria: Patients diagnosed as Diabetes on the basis of criteria laid down by American Diabetic Association (ADA) 2013, attending in and Out patient department of Subharti C.S.S.Hospital.

Exclusion Criteria: patients suffering from end organ diabetic complications.

5 ml of whole blood was collected in which 2 ml of blood was transferred for testing biochemical parameters & 3ml of blood was used for molecular study.

Molecular studies were done at Central Research lab of Subharti Medical College. Meerut. Peripheral venous blood was used as a source of total genomic DNA by kit method (Hi PurA™ Blood Genomic DNA mini-prep Purification kit) (Fig.1.1) The kit isolates the DNA from whole blood with spin column procedure. Quality and quantity of the extracted DNA was checked by Nano Drop 1000 spectrophotometer model no. picogene Genetix Biotech (India) (Fig.1.2). The polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method was used to genotype the G801A polymorphism in the SDF- 1β gene. The total volume

for PCR was 20µl which contained 50 ng of genomic DNA, 2µl of forward primer and 2µl of reverse primer, 10µl of taq premix & distilled water 25µl of mineral oil was added to PCR mix.

Forward primer 5' – CAG TCA ACC TGG GCA AAG CC – 3'

Reverse primer 5' – AAG TTT GGT CCT GAG AGT CC - 3'

The content was vortexed briefly and tube was placed in thermocycler 2720 Applied Biosystem (Singapore) for ampilification of DNA fragment. Initially DNA was denatured at 94° c for 5 min. This was followed by PCR (35 cycles) , with the following thermal cycling conditions : 30 sec at 58° c , 2 min at 72°c, then final extension for 5 min at 72° c in thermal cycler. (Fig. 1.3)

The PCR product was subjected to electrophoresis on ethidium bromide stain 4% agarose gel. Ampilified genomic DNA (302 bp) was digested by 5U of MSP1 restriction endonuclease and electrophoresed on ethidium bromide stain 2% agarose gel. Electrophoresis was performed at 120 V for 2 hrs in 50X TAE buffer. Ampilified products were visualized on a Gel Doc (Genei Merck). A 100 bp DNA ladder was used to verify sizes of the ampilified DNA fragments.(Fig 1.4)

Statistical Analysis: Hardy Weinberg equilibrium was assessed using genotype data. Allele and genotype frequencies were calculated in patients and healthy controls by direct gene counting. Statistical analysis of the differences between groups was determined by X^2 test and SPSS software version 10. Relative risk associated with genotype was estimated by the Odd ratio formula. P- Value of less than 0.05 was considered significant.

RESULTS

The ampilified PCR product of SDF - 1βgene was 302 bp in length. It covers the + 801 region. After digestion with restriction endonuclease enzyme, MSP1, it was to be separated into 210 bp and 212 bp fragments if it had a substitution of A to G at np + 801 to constitute the recognition site for MSP1.

For wild gene the 302 bp after restriction digestion yielded two bands at 100 and 202 bp. For heterozygous mutants(A/G), the bands were present at 302 , 202 and 100 bp. For homozygous mutant (A/A) there was only a single band at 302 bp.

Evaluation of the G801A polymorphism in SDF- 1β by MSP1 restriction endonuclease revealed that the frequency of the G/G genotype was 50 (33.3%) in patients and 15(10%) in healthy control. The prevalence of G/A genotypes was 75(50%) and 15(83%) in patients and controls respectively. The frequency of the A/A genotype in patients was 25(16.67%) and in controls, it was 20(13.3%). The frequency of G allele as calculated by hardy Weinberg equilibrium was 175 (58%) and 145 (48.3%) in diabetic patients, and controls respectively. When we calculate the A – allele, 125 (41%) were observed in diabetic patients, and the frequency of this allele was 155 (51%) in controls.

The odd ratio of SDF -1ß genotypes and alleles were calculated in T2DM and compare them with control subjects. The frequency of SDF -1 ß G / G homozygous wild in T2DM showed a significantly high relative risk, when compared with healthy controls. Relative risk associated with this genotype was 4.500. P value was calculated to be < 0.0001. The frequency of SDF 1- ß G / A heterozygous observed between T2DM patients and healthy controls which showed a significant negative relative risk of 0.304 , (P < 0.0001). Conversely the frequency of SDF -1 ß A /A homozygous mutant in patients with diabetes showed a relative risk of 1.300, which was not significantly associated with this genotype (p = 0.4197). The overall allelic frequency of SDF -1 ß G / G allele in either homozygous or heterozygous condition showed the relative risk to be 1.496 in T2DM patients compared to healthy controls with a p- value of 0.0143. (Table 1)

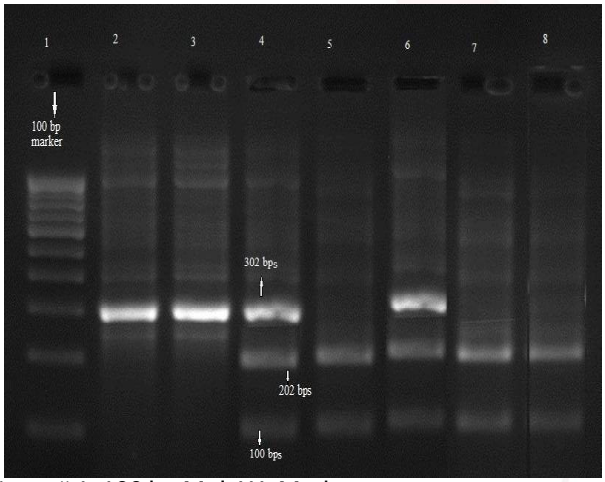
Table 1: Results of molecular studies.

Genotypes	T2DM (150)	Control (150)	OR (95% CI)	P- Value
G/G n (%)	50 (33.3 %)	15 (10 %)	4.5	P < 0.0001
GA n (%)	75 (50 %)	115 (83 %)	0.304	P < 0.0001
AA n (%)	25 (16.67 %)	20 (13.3 %)	1.3	P =0.4197
Alleles				
Gn (%)	175 (0.58)	145 (0.483)	1.4966	P =0.0143
An (%)	125 (0.41)	155 (0.516)	0.668	P =0.0143

Fig. 1.1: Photograph showing extraction of DNA.

Fig. 1.3: Photograph showing ampilification of DNA using thermocycler.

Fig. 1.5: PCR – RFLP results of SDF- 1ß Gene on 2.0% agarose gel.



Lane # 1: 100 bp Mol. Wt Marker.
Lane # 5, 7 & 8: G/G Wild type (2 bands at 202 and 100 bp)
Lane # 4&6: G/A heterozygous (3 bands at 302,202 and100 bp)
Lane # 2&3: A/A homozygous (a single band at 302 bp)

DISCUSSION

Diabetes has become a common global health problem that affects >170 million people worldwide. According to a study conducted by Wild et.al (2004) the top three countries with individuals suffering from T2DM are India (31.7 million in2000; 79.9 million in 2030) China (20.8 million in 2000; 42.3 million in 2030); and the U S (17.7 million in 2000; 30.3 million in 2030). So we are

not far from the day when India leads the World with largest number of diabetic subjects [12].

T2DM is a complex polygenic disorder in which common genetic variants interact with environmental factors to unmask the disease. Genetic factors are known to play an important part in the development of T2DM. However the role genetics plays in the development of diabetes is poorly understood.

Indian population provides a valuable genetic resource for mapping gene polymorphism as this population has still not been explored when comparing with other populations in advanced countries like Japan etc. As the objective of our study was to find the potential correlation of

Fig 1.2: photograph showing quantification of extracted DNA using picogene Genetix Biotech.

Fig. 1.4: Photograph showing bands of ampilified DNA on Gel Doc.

SDF - 1ß G801A polymorphism in T2DM patients in North Indian population, our study shows the association of SDF -1ß gene polymorphism in T2DM patients when compared to healthy controls. In the present study, we observed that the AA (mutant) genotypes of T2DM patients didn't show any statistical significant difference when compared with normal subjects, in the SDF -1 ß gene in the 801 region. In our study the frequency of this allele is more in controls (52%) than in T2DM patients (41%). Our results are similar to the results of the previous study conducted by Derakshan et al., on Iranian T2DM population where also the frequency of 'A' allele to be increased in healthy controls (16.3%) when compared to T2DM patients(15.3%) [13].

Our results also support the study of Vijay Viswanathan who showed the mutant 'A' allele to be protective allele for T2DM patients in South Indian population [14].

In our study , the "GG" (wild) genotype of SDF- 1ß showed a statistical difference when we compared the T2DM patients with healthy controls. The frequency of "G" allele is found to be increased in T2DM patients (58%) than in the control group (48%).'G' allele is actually considered as the wild form of SDF - 1ß gene in the 801 region. According to Derakshan et.al statistical analysis of allele did not exhibit a significant difference between patients and control. However our findings are in consistence with that of Vijay Viswanathan who showed that the frequency of 'G' allele was increased in T2DM patients (17%) than in the control group (4%).

We compare our study with the previous studies on SDF - 1ß gene polymorphism , our results are in consistence with the study done by Chaudhary et al., in North Indian population in high risk sero negative & HIV positive patients . They also found the frequency of wild 'G' allele to be increased in HIV patients (91.5%) when compared to healthy individuals (79.5%), while the frequency of mutant 'A' allele to be decreased in HIV – 1 positive patients (8.5%) as compared to healthy controls (20.5%), which shows 'A' allele to be the protective allele while 'G' allele to be the risk allele in HIV – 1 patients [15]. Similar findings were published by Vairaktaris et al on the association of SDF – 1 with the advanced stages of Oral cancer in Greek population. The author also concludes the mutant 'A' allele to be the protective allele that decreases with the stages of cancer.

The frequency ranges from 25.3% in control group to about 12.5% in advanced stages of disease (p= 0.005) [16].

CONCLUSION

On the basis of our study we can conclude that The "GG" genotype of SDF-1β gene is confined to be the risk allele and is associated with T2DM patients in North Indian population.

Although this study focused on a relatively small number of individuals, its finding contribute to the growing evidence of the presence and effects of genetic variant in the understudied North Indian population. The increase in the incidence of T2DM throughout the world compels the need to understand the disease etiology to develop strategies that might slow the trend of increasing incidence of T2DM, and to identify new therapeutic approaches.

Conflicts of Interests: None

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