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Mitochondrial DNA Single Nucleotide Position16189 Polymorphism and Type 2 Diabetes in Central Indian Population

S. S. Bandyopadhyay[1] and G. R. Lakshmi[2]

ABSTRACT

The study investigated the role of mitochondrial DNA mutation for the development of type 2 diabetes through a case control study of Indian population in Central India. We have attempted to estimate the extent of involvement of mitochondrial DNA mutation (16189) from T>C and in and around poly c tract (nucleotide position 16180 to 16194 of non coding region) and its association with coding region (nucleotide position 3169 – 6031) for the prognosis of type 2 diabetes in Central Indian Population.

INTRODUCTION

Type 2 Diabetes is a complex heterogeneous group of condition characterized by elevated level of plasma glucose. The recent global epidemic of type 2 diabetes is an indicative of environmental triggers. Multiple lines of evidence support the view that genetic components play a crucial role in the prognosis of type 2 diabetes in which polymorphism in mt DNA might have played a role both through genetic and environmental mutagens. According to the recent projection, India already leads the world with the largest number of diabetic subjects and predicted that by 2030 there will be 80 million(Wild et al .2004) . Insulin has been shown to enhance muscle mitochondrial biogenesis in human. In people with type 2 diabetes, increasing insulin from the post absorptive to post-prandial level does not increase ATP production unlike in non-diabetic people (Muoio and Koves. 2007). The uncoupling of beta oxidation of fatty acids and the TCA cycle, which is induced by chronic inactivity and a high fat diet, cause both insulin resistance as

[1] and [2] Anthropological Survey of India, Nagpur.

well as intra-myocyte accumulation of partially oxidized lipids from skeletal muscle (Stump et. al.2003).

India is facing an epidemic of type 2 diabetes with high prevalence in urban area. Various epidemiological studies in India have shown that the increasing prevalence of diabetes could be attributed to a high genetic risk, both from nuclear and mitochondrial DNA and lower risk thresholds for acquired risk factors such as age, obesity, abdominal adiposity (Ramachandran et al.2004 and Ramachandran et al. 2006). Mitochondrial dysfunction has been reported to be crucial to the pathogenesis of non alcoholic fatty liver disease (Wei et al. 2008). Non-alcoholic fatty liver disease has been found to be more in Asian Indian men (Peterson et al. 2006).

The recent study (Nair et al .2008) among non-resident Asian Indian diabetic, Asian Indian non diabetic and northern European American non diabetic, for mitochondrial dysfunction, insulin sensitivity, intramuscular triglyceride, lipid profile, proinflamatory markers like interleukin 6 (IL–6), C – reactive protein and Tumor Necrosis Factor – alpha, concluded that Indian diabetic males have more insulin resistance, higher mitochondrial copy number, higher ATP production rate compared to Northern European non diabetic individuals. Thus, it was concluded that among the Asian Indian, insulin resistance and mitochondrial dysfunction may be unrelated and intra muscular triglyceride (IMTG) were higher in non diabetic Indians compared to the non diabetic Northern European Americans.

Multiple pathogenic pathways are able to deregulate glucose homeostasis leading to diabetes variants in mitochondrial DNA (mt DNA) may contribute to the pathophysiology of type 2 diabetes (Liou et al. 2007). The mutation 3243A>G leads to the development of diabetes due to an inappropriate storage of triglycerides with in adipocytes and inappropriate scavenging of fatty acids by _-oxidation (Maassen et al. 2006). A transition of T>C at nt position 16189 in mitochondrial DNA has attracted bio-medical researchers for its probable role with the development of diabetes mellitus in the adult life (Liou et al. 2007).Notably the variant at 16189 T>C was more prevalent with left ventricular hypertrophy (LVH) than without LVH in diabetic patients (Momiyama et al. 2003).

The role of mitochondria in causing disease is largely attributed to its reactive oxygen species (ROS) production. The suggestive role of mt DNA variants in increasing ROS production and the impaired response to oxidative stress due to T 16189 C variant is worth addressing as genetic susceptibility factors in type 2 diabetes mellitus (Wallac1992, Lin et al.1994 and Bhat et al. 2007) Mitochondrial DNA mutations are an important cause of many diseases including diabetes (Wallace1992 and Chinnery et al.1997). Patients harboring mt DNA mutation usually have a mixture of mutated and wild type

(normal) mt DNA (hetroplasmy). In vitro studies have shown that hetro plasmic mt DNA defects are only expressed when the percentage level exceeds a critical threshold level (Larsson and Clayton 1995). The level of mutated DNA and mt DNA (mutation load) varies both between and within individuals with mt DNA disease (Lightowlers et al. 1997). This variability, coupled with tissue specific differences in the threshold of expression, partly explains the diverse clinical phenotypes, which are seen in patients harboring the same mt DNA defect (Wallace 1992).

The T>C substitution at nt position 16189 of the human mitochondrial genome has been associated with the development of hetro plasmic length variation in the control region of the mitochondrial DNA. This defect may be due to pathogenic mt DNA mutation including the diabetogenic A>G mutation in the t RNA (Marchington et al. 1996). 16189 mt variants have also been associated with insulin resistance in British adult men. Mt DNA – NDI gene mutations at nt 3243 (A>G), nt 3316 (G>A), nt 3318, nt 3394 (T>C) and nt 3426 (A>G) may contribute to the pathogenic environmental factors (McCarthy et al .1996, Odawara et al. 1996, Maassen et al. 2004 and Hattori et al .2003). Early age at onset and maternal inheritance are risk factors for diabetes mellitus caused by mitochondrial DNA – NDI gene dysfunction (Pei et al. 2004) .The mt DNA 16189 variant can influence the development of type 2 diabetes mellitus. The association between the 16189 variant and increased BMI exemplify an additive effect of genetic and environmental factors on the pathogenesis of type 2 diabetes mellitus. The prevalence of the 16189 variant is higher in type 2 diabetes mellitus patients than in the age and sex matched non-diabetic subjects (Weng et al .2005).

A transition of T>C at nt position 16189 in the hyper variable D-loop region of mt DNA has attracted research interest for its probable correlation with increasing insulin resistance and development of diabetes mellitus in adult life (Liou et al. 2004). The 16189 variant arises when a T16189C transition results in a poly-c tract in the large non-coding region of mt DNA, near to control sequences for replication and transcription. The majority of UK Caucasian with the variants is homoplasmic for the T16189C transition with a length of 10C residues. This may generate a heteroplasmic length variation, each individual having a range of tract length including a majority of mt DNA with tract length of 9 and 11. Hetro plasmic length variation of homo polymeric tract becomes more pronounced with tracts whose model length is over 10C (Poulton et al.2002). The genetic variation of the 16184 – 16193 poly C tract is unlikely to have a major role in the cause of type 2 diabetes (Chinnery et al. 2005). A multinational population based case control study confirmed the association between the 16189 variants and type 2 diabetes among Chinese, Japanese and Koreans (Park et al. 2008).

In the present communication, we have attempted to estimate the extent of involvement of mitochondrial genetic factors (16189 T > C mutation, poly C tract 16180 – 16194) involvement in non coding region, its association with coding region (3169 – 6031) for the prognosis of type 2 diabetes in central Indian population. The study investigated the role of mt DNA mutation for the development of type 2 diabetes through a case control study.

MATERIAL AND METHOD

The study was performed in accordance with the declaration of Helsinki and formally approved by the institutional ethics committee of Anthropological Survey of India, Kolkata, India. Informed consent was obtained from all subjects. According to World Health Organization diagnostic criteria of diabetes mellitus (1999), we have enrolled 226 persons out of which 60.4% were diabetic and the rest were non diabetic 39.6%. Data from the subjects include gender, age, age at onset of diabetes, height, weight, circumference at hip and abdomen, systolic and diastolic blood pressure were recorded in a structure schedule from a polyclinic, Nagpur, Maharashtra (India) for the diabetic patients and home visit for the controlled subjects. Diabetic complications along with other relevant information were carefully recorded from the record of the patients and through personal interview. 2ml blood samples were collected in BD vacutainer by vein puncture and 4 ml of blood was collected from each subject for the extraction of DNA in BD vacutainer containing K2 EDTA 7.2 mg (B. D. Franklin, NU, USA). Fasting blood sugar was recorded by strips method using a glucometer (one touch, Ultratech). The levels of total cholesterol and triglyceride were estimated by semi-automated photo analyzer mini techno 1SE SRL, Roma, Italy) from serum samples.

The DNA was extracted from the blood by salt precipitation followed by Proteinase K treatment. OD was determined by vis-UV Spectrophotometer (UV 1601 Shimadzu Corporation, Japan).The mitochondrial DNA was amplified by long polymerase chain reaction (PCR) using specific primer for particular region of mitochondrial DNA (Table-1). The reaction mixture (10 µl) containing 14 p Mol of each of primer, 0.4 µl of 0.2 m M each of de oxy nucleotide triphosphate, 2.5 m M Magnesium chloride and 0.6 unit of Taq DNA polymerase, 1 µl of PCR buffer (10 X PCR buffer). The final volume was made up to 10 µl with pre-sterilized mille-Q-water. The amplification protocol consisted of an initial denaturation at 94°C for two minutes followed by 35 cycles of denaturation at 95°C for one minute. Annealing at 60 C for 45 seconds and extension at 72 °C for 3.20 minutes with a final extension at 72°C for 7 minutes using gene Amp PCR System 9700 (Applied Bio system). The sequencing of the sample was done in 3700 Genetic Analyzer of Applied Bio system using Big dye (R) Terminator V 3.1 cycle sequencing kit (Applied

Bio system, Foster City, CA 94404, USA), following the suppliers instructions with certain modifications.

Table : Primer sequence for different position of mt DNA

Sl. No	Primer	Position	5' Sequence 3'
01.	3169F	3136 – 3189	TACTTCACAAAGCGCCTTCC
02.	3961R	3961 – 3941	ATGAAGAATAGGGCGAAGGG
03.	3796F	3769 – 3816	TGGCTCCTTTAACCTCTCCA
04	4654R	4654 – 4634	AAGGATTATGGATGCGGTTG
05.	4485F	4485 – 4505	ACTAATTAATCCCCTGGCCC
06.	5420R	5420 – 5400	AATGGGGTGGGTTTTGTATG
07.	5255F	5255 – 5275	CTAACCGGCTTTTTGCCC
08.	6031R	6031 – 6011	ACCTCGAAGGTTGCCTGGCT
09.	15811F	15811 – 15831	TCATTGGACAAGTAGCATCC
10.	16536R	16536 – 16516	GAGTGGTTAATAGGGTGATAG

RESULTS

Out of total sample of 226, 138 were diabetic and rests were non diabetic without any observable complications reported. There were 54 samples whose in and around poly C tract (G>A), i.e. 16180 – 16194, changed nucleotides sequence were noticed in any of the position as determined by direct sequencing. Out of 54 samples, 30 samples were with the transition from T>C in nt position 16189. Fifty percent of the nt 16189 mutated samples out of 36 were diabetic. The mean age is 45 years for controls and 55 years for diabetic. The average age at onset of diabetes is 49 years. The polymorphism of around poly-C tract has been depicted for 226 readable sequences out of which 76.11% are of wild type i.e. matched with the revised sequence of Cambridge and 13 polymorphism were observed in the sequence. Among 7.52% samples, T > C has been observed only at position 16189, 23.89% samples were found with changed sequence of in and around poly-C tract. Out of 15.93% samples, where nt 16189 T>C variation occurred, 50% were diabetic samples, whereas among the poly c tract disturbed samples, 55% samples were diabetic. Considering the total samples of 226, among 60.55 diabetic samples, 13.27% resulted from poly-C tract disturbance and 8% from nt 16189 T>C mutated samples. In the wild type sequence, there were 47.79% diabetic and 28.32% non diabetic. Out of 16189 mutated samples, 50% were diabetic (18/36) and in the poly-C tract mutated samples, 55.56% (30/54) were diabetic.

The samples were classified in three categories i.e. (1) 16189 (C>T) mutated, (2) Poly-C tract mutated and (3) wild type where no mutation occurred in 16189 (C>T). The poly-C tract mutated samples include 16189 (C>T) mutated samples and two groups i.e. Diabetic and control.

Table : Relative frequencies of polymorphic mt DNA sequences between nucleotide position 16180 to 16194

Type	Nucleotide position from 16180 – 16194															Frequency	Percentage
	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94		
Wild	A	A	A	A	C	C	C	C	C	T	C	C	C	C	A	172	76.106
Mutated	A	A	A	A	C	C	C	C	C	C	C	C	C	C	A	17	7.522
Mutated	A	A	A	C	C	C	C	C	C	C	C	C	C	C	A	7	3.097
Mutated	A	A	C	C	C	C	C	C	C	C	C	C	C	C	A	6	2.654
Mutated	A	A	A	A	T	T	C	C	C	C	C	C	C	C	A	1	0.442
Mutated	A	A	A	A	C	C	C	C	C	C	C	C	T	C	A	3	1.327
Mutated	A	A	A	A	C	C	C	C	T	T	C	C	C	C	A	5	2.212
Mutated	A	A	A	A	C	C	C	C	C	T	C	C	T	C	A	7	3.097
Mutated	A	A	A	A	C	T	C	C	C	T	C	C	C	C	A	3	1.327
Mutated	A	A	A	A	C	C	C	T	C	T	C	C	C	C	A	1	0.442
Mutated	A	A	A	A	C	C	C	C	C	T/C	C	C	C	C	A	2	0.884
Mutated	A	A	A	A	T	C	T	C	C	C	C	C	C	C	A	1	0.442
Mutated	A	A	C/A	C/A	C	C	C	C	C	T/C	C	C	C	C	A	1	0.442
TOTAL																**226**	**100.00**

Table : Characteristics, prevalence of mutation at different nucleotide position groups

Condition	Position (Shift)	Risk Ratio	95% Confidence Internal	Odds Ratio	95% Confidence Internal	t Value
16189 (C–T)	3316 (G>A)	1	0.068 – 14.787	1	0.058 – 17.326	0.757
Poly C Tract Mutated		1.6	0.154 – 16.605	1.643	0.140 – 19.288	0.585
Wild type		0.594	0.086 – 4.132	0.589	0.081 – 4.283	0.479
Wild type						
16189 (C–T)	3531 (G>A)	0	0	0	0	0
Poly C Tract Mutated		0	0	0	0	0
W		2.982	0.356 – 24.965	3.077	0.352 – 26.936	0.272
16189 (C–T)	3630 (C–T)	1	0.231 – 4.310	1	0.173 – 5.772	0.671
Poly C Tract Mutated		3.2	0.382 – 26.782	3.539	0.369 – 33.978	0.253
Wild type		0	0	0	0	0
16189 (C–T)	3741 (C–T)	0	0	0	0	0
Poly C Tract Mutated		0.8	0.053 – 12.137	0.793	0.047 – 13.377	0.696
Wild type		0.596	0.179 – 1.982	0.577	0.161 – 2.074	0.298
16189 (C–T)	3921 (C–T)	0	0	0	0	0
Poly C Tract Mutated		0.8	0.053 – 12.137	0.793	0.047 – 13.377	0.696
Wild type		2.982	0.674 – 13.188	3.182	0.675 – 15.002	0.107

Condition	Position (Shift)	Risk Ratio	95% Confidence Internal	Odds Ratio	95% Confidence Internal	t Value
16189 (C–T)	3954 (C–T)	0	0	0	0	0
Poly C Tract Mutated		0.8	0.053 – 12.137	0.793	0.047 – 13.377	0.696
Wild type		2.385	0.272 – 20.885	2.438	0.267 – 22.298	0.381
16189 (C–T)	4216 (C–T)	0	0	0	0	0
Poly C Tract Mutated		0.8	0.053 – 12.137	0.793	0.047 – 13.377	0.696
Wild type		0.994	0.246 – 4.023	0.996	0.230 – 4.302	0.631
16189 (C–T)	4796 (C–T)	0	0	0	0	0
Poly C Tract Mutated		3.2	0.382 – 26.782	3.539	0.369 – 33.978	0.253
Wild type		0	0	0	0	0
16189 (C–T)	4916 (G–A)	0	0	0	0	0
Poly C Tract Mutated		1.6	0.154 – 16.605	1.643	0.140 – 19.288	0.585
Wild type		1.193	0.225 – 6.332	1.2	0.214 – 6.741	0.60
16189 (C–T)	4917 (A–G)	0	0	0	0	0
Poly C Tract Mutated		0	0	0	0	0
Wild type		2.385	0.272 – 20.885	2.438	0.267 – 22.298	0.381
16189 (C–T)	5252 (A–G)	0	0	0	0	0
Poly C Tract Mutated		0	0	0	0	0
Wild type		0.149	0.017 – 13.305	0.141	0.015 – 1.292	0.065
16189 (C–T)	5360 (A–G)	0	0	0	0	0
Poly C Tract Mutated		0.8	0.053 – 12.137	0.793	0.047 – 13.377	0.696
Wild type		0.795	0.184 – 3.441	0.787	0.171 – 3.634	0.523
16189 (C–T)	5460 (G–A)	0	0	0	0	0
Poly C Tract Mutated		0	0	0	0	0
Wild type		0.239	0.048 – 1.194	0.224	0.042 – 1.192	0.069
16189 (C–T)	5744 (G–A)	0.50	0.284 – 7.934	1.6	0.234 – 10.945	0.5
Poly C Tract Mutated		1.60	0.320 – 8.007	1.692	0.283 – 10.135	0.448
Wild type		0	0	0	0	0
16189 (C–T)	5790 (C–A)	0	0	0	0	0
Poly C Tract Mutated		0	0	0	0	0
Wild type		0.596	0.124 – 2.868	0.585	0.115 – 2.988	0.4

Sequencing of mitochondrial DNA samples from nucleotide position 3169 to 6031 in the coding region and 15811 to 16536 in non-coding region was done. We have observed fifteen point mutations within this region of mitochondrial DNA where more than 2 percent samples were mutated from the Cambridge sequence of reference. Among the nucleotide 16189 (C>T) mutated samples at nt position 3316 (G>A), 3630 (C>T) and 5744 (G>A), mutations were observed with odds ratio one between diabetic and control subject (Table -3). Among the poly C tract mutated samples on nucleotide position 3630 (C>T), 4797 (C>T), 4916 (G>A) and 5744(G>A) the odds ratio is more than one among diabetic and control groups. The level of TG is significantly higher among the diabetic groups compared to control groups of poly C tract mutated and non mutated groups.

Whereas the comparison of TG level between 16189 (T>C) diabetic and control groups showed non significant results (student's t value is less than 1.96 i.e. $p<0.05$). Thus, nt position 16189 and poly C tract mutation may have impact in biogenesis of diabetes along with other point mutation in the coding region.

Out of the nine positions in the coding region where mutations have been observed with odds ratio more than one, we have observed eight positions are in the part of oxidative phosphorylation enzyme system. The position nt 3316 to nt 3954 are in the enzyme NADH dehydrogenase 1 and 4796 to 4916 are in NADH dehydrogenase 2. The nt position 5744 is a part of the L–Strand origin (Table 4).

Table : Coding region and nucleotide position mutated in different groups

Sl. No.	Coding region (nt position)	Sample type observation		
01.	**NADH dehydrogenase**	**16189 (T–C)**	**Poly C tract mutated (nt)**	**Wild Type**
	3316	Yes	—	—
	3531	—	—	Yes
	3630	Yes	Yes	—
	3741	—	Yes	—
	3921	—	—	Yes
	3954	—	—	Yes
02.	**NADS Dehydrogenase**			
	4796	—	Yes	—
	4916	—	Yes	—
03.	**L Strand Origin**			
	5744	Yes	Yes	—

Analysis of the anthropometric and other information revealed that the mean age of the three groups of control subjects is 45 years and that of the diabetic patients is 55 years (Table – 5).

Table : Comparison of Bio anthropometric parameters of the diabetic and control subjects (Mean ± SD) for different groups

Genetic Status of Subjects (Count)	Parameters (Mean ± SD)										
	Age (years)	Age at onset (years)	Systolic Blood Pressure	iastolic Blood Pressure	Fasting Sugar	Cholesterol	TG	Weight ratio	Statues	BMI	Waist/hip
Changed poly C tract (24) (control)	45.38 ± 15.86	—	125.57 ± 9.51	78.78 ± 6.02	89.20 ± 7.29	191.53 ± 31.58	143.50 ± 45.72	59.87 ± 12.57	157.79 ± 10.78	24.38 ± 4.68	0.88 ± 0.06

Genetic Status of Subjects (Count)	Parameters (Mean ± SD)										
	Age (years)	Age at onset (years)	Systolic Blood Pressure	iastolic Blood Pressure	Fasting Sugar	Cholesterol	TG	Weight ratio	Statues	BMI	Waist/hip
Changed poly C tract (30) (Diabetic)	55.63 ± 8.54	49.79 ± 9.46	144.13 ± 21.21	89.13 ± 11.53	142.10 ± 54.49	192.16 ± 47.01	182.16 ± 79.47	63.50 ± 9.13	156.64 ± 6.77	25.90 ± 3.62	0.82 ± 0.29
16189 T >C (18) (Control)	46.22 ± 15.18	—	125.29 ± 8.74	78.94 ± 5.25	89.00 ± 8.41	190.86 ± 33.19	143.40 ± 40.11	60.29 ± 12.87	157.17 ± 11.13	24.39 ± 4.84	0.88 ± 0.06
16189 T >C (18) (Diabetic)	55.94 ± 7.30	49.76 ± 8.46	141.44 ± 20.94	88.67 ± 11.68	134.22 ± 43.45	190.39 ± 52.67	173.53 ± 82.89	63.22 ± 9.21	156.77 ± 5.64	25.75 ± 3.73	0.78 ± 0.6
Wild Type (64) (Control)	45.86 ± 15.18	—	125.97 ± 15.04	81.86 ± 7.65	92.60 ± 12.43	201.04 ± 56.54	131.87 ± 60.60	63.39 ± 11.88	160.72 ± 8.61	24.51 ± 4.05	0.89 ± 0.08
Wild Type (108) (Diabetic)	56.54 ± 8.85	49.42 ± 9.38	139.20 ± 18.52	86.56 ± 12.02	142.12 ± 45.34	197.18 ± 50.36	180.85 ± 78.00	61.70 ± 8.73	154.79 ± 7.16	25.81 ± 3.69	0.92 ± 0.07

The mean age at onset of diabetes is 49 years. The systolic and diastolic blood pressure of the three control groups were 125 mm/Hg and 78 to 81.89 mm/Hg, whereas all diabetic patients showed higher values ranges from 139.20 to 144 mm/Hg and 86 to 89 mm/Hg. The fasting blood sugar of the three control groups showed a value of 89.20 to 92.60 mg/dL and that for diabetic patients show higher range (134.22 to 144.12 mg/d L), which is 61.89% higher than the control groups. The mean post prandial blood sugar was below 150 mg/d L for control subjects and it was above 220 mg/d L for diabetic patients of all three groups after two hours of normal meal. The serum cholesterol level of the three diabetic and control groups ranges between 191 to 201 mg/d L. No substantial variation has been observed between diabetic and control groups. The serum triglyceride value of the control groups ranges from 131.87 to 143.40 mg/d L and that of the diabetic groups between 173.53 to 182.16 mg/d L, which is higher in all the diabetic groups than the control groups. The five point mutations (nt 3316, 3630, 4796, 4916, 5744) were observed with odds ratio more than one between diabetic and control subjects. The wild type i.e. where no mutation observed in the poly C tract area, we have encountered three point mutation at nt position 3531 (G>A), 3921 (C>T) and 3954 (C>T) with odds ratio more than one. The highest odds ratio of 3.539 have been observed at nt position 3630 (C>T) and 4796 (C>T) among the poly C tract mutated samples.

The student's t test between diabetic and control samples where no mutation taken place in poly C tract area of mt DNA, significant differences found in SBP, DBP, FBS and TG (Table – 6).

Table : t' Test between diabetic and control subjects among different groups for Physiological, Biochemical and Anthropological Parameters

Genetic Status of Subjects	Age	SBP	DBP	Fasting Blood	Cholester of	TG	Weight	Statues	BMI ratio	Waist/hip
16189 T >C	2.51	3.10	3.31	4.48	0.03	1.43	0.81	0.14	0.97	1.17
Mutated Poly C tract	2.91	4.36	4.33	5.36	0.06	4.01	1.21	0.46	1.34	0.32
Without Poly C tract mutation	5.71	5.07	3.14	8.84	0.71	3.90	1.26	4.50	1.76	3.27

In case of poly C tract mutated samples, significant differences exist between diabetic and control subjects in SBP, DBP, FBS and TG. Similarly' test in case of nt 16189 (T>C) mutated diabetic and control subjects show significant differences in all three categories for SBP, DBP and FBS, where as no significant difference exist for cholesterol between all the three categories of diabetic and control groups. Student's t test of anthropometric parameters, ratios and indices show significant differences in stature and waist hip ratio of diabetic and control subjects where no mutation occurred in poly C tract.

Discussion

The current study demonstrated that the mean values of anthropometric parameters are higher among the diabetic samples compared to control samples in all the three categories. Thus, the phenotypic expression in the level of carbohydrate and fat metabolite product i.e. glucose, cholesterol, T G are higher in diabetic compared to control subjects. The intake of rich carbohydrate, less protein and fat diet is indicative of the fact that cholesterol level is not significantly different between diabetic and control subjects. It indicates that the intake of free fat, which is the source of cholesterol, is same in both diabetic as well as control. In this studied population, the increased level of TG in diabetic compared to controls are indicative of involvement of fat accumulation as well as glucose accumulation in the blood plasma.

BMI and waist/hip ratio are indicative of fat deposition, show higher mean values for the poly C tract mutated groups as well as in nt 16189 (T>C) mutated diabetic patients than the controls. Food intake of all subjects is uniform as they are fixed income salaried people and many lead sedentary lives. Their energy requirement and intake remains almost constant throughout the day. Seasonal diet change can not be expected from this studied population. Thus, the thrifty gene hypothesis of Neel (1962) can not be applied to this population for prognosis of diabetes due to maximum metabolic efficiency of gene and fat storage as less number of obese people was found among the diabetic patients. The other hypothesis (Wei et al 1998) of organ specific origin cannot be completely ruled

out as the maintenance of glucose level within a reasonable limit does not affect the organ function within a detectable level. The third theory of Mendelian origin (Lin et al 1994)of diabetes cannot be fully ruled out as most of the cases have some diabetic history attached directly or distantly. However, all diabetic patients do not fallow this hypothesis. Thus, environmental trigger of diabetes is the best possible hypothesis which encompasses both genetic and no genetic components applied to these subjects .The genetic components change by chemical mutagenesis is the best possible target to explain the prognosis of diabetes. As diabetes is the over production, accumulation, faulty absorption and delayed catabolism rate in single or in a synergic way alters the glucose hemostasis. Mean body weight of the diabetic patients of poly C tract mutated sample was higher than the control group where as the same parameter was higher in control group of wild type sample compared to diabetic group. The average body mass index values ranges from 24.38 to 24.51 for three control groups where as that for diabetic groups the values were above 25, which belong to over weight category. The waist/hip ratio of the poly C tract control groups were higher than that of the diabetic group of the poly C tract, however, for wild type samples, reverse trend has been observed between control and diabetic samples.

In the present study we could not find statistically significant difference of BMI and WHR between diabetic vs. control and between 16189 mutated vs. Poly C tract mutated samples. However, we have observed a significant difference between diabetic and control subjects for non poly C tract mutated samples. Our findings collaborate with the finding of Korean people (Chinnery et al 1997).

Screening for point mutation in the NDI region at nt 3316, 3531, 3630, 3741, 3921 and 3954 position suggested that a large number of the diabetes patients who were diagnosed after the age of 45 years reported mutation in the NADH1 region, which is consistant with the hypothesis that oxidative strees induced by mitochondria increases with aging (Kamiya and Aoki 2003). Mitochondrial DNA unlike nuclear DNA, protected by histamine, is more sensitive to be attacked by reactive oxygen species produced by mitochondria and susceptible to mutation, which may be acclerated in the diabetic state (Lin et al. 2006). It has been suggested that increased mitochondrial reactive oxygen production (OA-, H2O2) during hyperglycemia may contribute to the pathology of diabetes.

In our study the frequency of diabetes patients is 50% of the total samples with 16189 (T>C) mutation. 60% of the samples with poly c tract disturbed have diabetes. Harboring the 16189 mt DNA variant may impair the ability of a cell to respond properly to oxidative stress and oxidative damage as suggested earlier (Wei et al. 1998) holds good for the present study. The increase of BMI, WHR and TG in diabetic state compared to normal state is possibly the result of variation in the mt DNA sequence, though other genetic (hereditary and non-hereditary) and non-genetic factors may contribute for precipitation to diabetes. Variation in the individual and regional predisposition to degenerative disease like diabetes may result from the interaction of intake of carbohydrate loaded vegetarian

food in Indian diet due to excess production of reactive oxygen species, in time and space. Therefore the mitochondria provide a direct link between environment and genes in this population.

The mitochondrial DNA, although not protected by histones or DNA binding proteins, is susceptible to oxidative damage by ever increasing levels of ROS and free radicals in the mitochondrial matrix with increase of age and pathogenesis of degenerative diseases (Wei et al.1998, 2001) .In Indian diet rich in carbohydrate increases the chance of production of more ROS thus increases the chance of unprotected mitochondrial DNA damage. Nucleotide position 16189 is very near to control region which directs the production of the mitochondrial DNA. Thus, mutated mitochondrial DNA decreases the production of correct mitochondrial DNA quantity responsible for the underutilization of glucose, which in turn leads to hyperglycemia. In this study our findings demand further study with greater sample size to eliminate the effect of genetic factors of nuclear DNA origin and specific enhancer of ROS mutagens which increases the chance of occurrences of diabetes in this population. Nucleotide position 16189 mutations in mitochondrial DNA can be taken

as a marker for development of type 2 diabetes in Indian population as in other Asian countries.

Declaration

The views expressed in this paper are completely of the authors

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