

Original Research Article

Study of DNA Methylation in offsprings of Type 2 Diabetics

Abstract:

Introduction: Epigenetic is study of changes in gene expression that occur by changing the by modifying DNA methylation and remodeling chromatin. It may also be affected by the environment, thus an important pathogenic mechanisms in complex multifactorial diseases such as type two diabetes. Recent genome-wide association studies have identified a number of genetic variants that explain some of the inter individual variation in diabetes susceptibility. Thus keeping in view the role of epigenetic mechanisms particularly DNA methylation the present study was conducted to find out DNA methylation (Whole DNA) and its association with Type 2 Diabetes mellitus.

Methods: For the present study, the families of one hundred Diabetic individuals were enrolled. All the off springs of diabetics above the age of 18 years were recruited for the present study. All the samples collected were analyzed for Fasting plasma glucose, Glycosylated Hemoglobin, Lipid profile complete and DNA methylation (5mcytosine)

Results: The study included 100 diabetic individuals and their off springs in the age range of 18-35 years. 5mc% was more in IFG and Diabetic individuals as compared to normal males and females.

Conclusions: Changes are occurring at an early age i.e. 18-24 years, increased DNA Methylation in these individuals indicates towards deranged metabolism and development of inflammation in these individuals leading to impaired fasting glucose and making them more prone to type 2 Diabetes Mellitus at a later age.

Key Words: Diabetes Mellitus, Offsprings, Epigenetics, DNA Methylation

UNDER PEER REVIEW

Introduction:

Diabetes mellitus is one of the most common non-communicable metabolic disorders which is manifested by hyperglycemia, resulting due to defects in insulin secretion, insulin action or both. The incidence of diabetes mellitus is increasing along with increased proportion of individuals with impaired fasting glucose or pre diabetes. The disease formerly considered to be affecting middle aged is incident now in young and adolescent as indicated in previous studies.

Although there is no uniform definition of Epigenetics, it is defined as a heritable change in gene function that occurs without a change in nucleotide sequence. Epigenetic is presently described as the study of changes in gene expression that occur not by changing the DNA sequence¹, but by modifying DNA methylation and remodeling chromatin. In recent years, major advances in the understanding of epigenetic mechanisms have established them as key players in several cellular processes including cell differentiation, aging, DNA replication, and repair²⁻⁵.

These modifications, including acetylation, methylation, phosphorylation, and ubiquitination, alter the interaction between the histones, DNA and nuclear proteins, therefore affecting gene transcription and regulate gene silencing or expression⁶. Epigenetic effects may also be affected by the environment, making them potentially important pathogenic mechanisms in complex multifactorial diseases such as type 2 diabetes. DNA methylation is commonly associated with gene silencing and contributes to X chromosomal inactivation, genomic imprinting and transcriptional regulation of tissue- specific genes during cellular differentiation.

Although obesity, reduced physical activity, and aging increase susceptibility to type 2 diabetes, many people exposed to these risk factors do not develop the disease. Recent genome-wide association studies have identified a number of genetic variants that explain some of the inter individual variation in diabetes susceptibility⁶⁻¹⁰.

Thus keeping in view the role of epigenetic mechanisms particularly DNA methylation the present study was conducted to find out DNA methylation (Whole DNA) and its association with Type 2 Diabetes mellitus.

Material and Method: The present study was conducted in Multidisciplinary Research unit GMC Amritsar in Association with Department of Biochemistry and Medicine at Government Medical College and Guru Nanak Dev Hospital Amritsar. The study was conducted after approval from the Ethical committee (Code No GMCIEC00095). For the present study, the families of one hundred Diabetic individuals were enrolled after obtaining informed consent. All the off springs of diabetics above the age of 18 years were recruited for the present study. Sample collection was done by calling the family members in outpatient department. All the members were advised to observe at least 8 hours fast. To comply with the instructions the sample collection was started at 7.30 am in the morning. Samples collected were labeled using a number code for a family. All the samples were transferred to multi disciplinary research unit for serum separation, processing and storage for further analysis. All the samples collected were analyzed for

1. Fasting plasma glucose¹¹
2. Glycosylated Hemoglobin¹²
3. Lipid profile complete (Total cholesterol¹³, HDL¹⁴, LDL¹⁵, VLDL and Triglycerides¹⁶)

The samples were stored at -70⁰C to estimate DNA methylation (5mcytosine¹⁷) DNA was isolated by using Standard Phenol Chloroform method described by Adeli and Oqbonna 1990¹⁸ and quantification of DNA was done using Agarose Gel Electrophoresis method as described by (Sambrook et al 1989)¹⁹. After quantification the methylation of DNA was estimated using commercially available ELISA kits.

The data thus generated was analyzed for statistical significance using one way ANOVA and SPSS version 19.0. Pearson's co-efficient of correlation was used to calculate the correlations amongst various numerical parameters and Chi square was used to calculate correlation between nominal variables. $p < 0.05$ was considered statistically significant.

Results: The study included 100 diabetic individuals and their off springs in the age range of 18-35 years. All the off springs of Diabetic individuals were sub divided into three groups depending on their age i.e. Group I 18-24 years, Group II >24-30 years and Group III >30-35 years. The maximum number of individuals belonged to age group II i.e. >24-30 years of age and were segregated according to gender i.e. males and females. Further all the individuals i.e. males and females were Normal, having IFG or Diabetic depending on the levels of fasting plasma glucose and glycated hemoglobin as per ADA criteria. Some of the individuals gave the history of being diabetic. All the individuals were evaluated for fasting plasma glucose, glycated hemoglobin, lipid profile and DNA methylation. The observations thus made are reported as under.

Table 1 Comparison of Fasting Plasma Glucose and Glycated Hemoglobin in Normal IFG and Diabetic Individuals belonging to different Age groups

Age Group	Gender	Glucose mg% (Mean \pm S.D)			Glycated Hemoglobin % (Mean \pm S.D)		
		Normal	IFG	diabetic	Normal	IFG	diabetic
18-24 years	Male	84.7 \pm 1.2	111.7 \pm 7.4**	160.4 \pm 2.9** \ddagger	4.47 \pm 0.55	5.8 \pm 0.35**	6.47 \pm 1.46** \ddagger
	Female	82.37 \pm 1.3	109.7 \pm 5.6**	\ddagger 201.85 \pm 7.9**	4.35 \pm 0.48	5.87 \pm 0.40**	6.71 \pm 1.61** \ddagger
>24-30 years	Male	84.0 \pm 1.3	112.85 \pm 6.7**	170.18 \pm 3.2**	4.41 \pm 0.6	5.85 \pm 0.26**	6.4 \pm 1.09** \ddagger
	Female	81.38 \pm 1.32	110.72 \pm 7.6**	\ddagger 195.05 \pm 8.17**	4.35 \pm 0.52	5.75 \pm 0.64**	6.39 \pm 1.28** \ddagger
>30-35 years	Male	86.11 \pm 1.22	111.4 \pm 7.9**	180.46 \pm 3.36** \ddagger	4.38 \pm 0.52	5.8 \pm 0.51**	5.8 \pm 1.22
	Female	79.31 \pm 1.33	109.79 \pm 6.56**	198.19 \pm 8.8** \ddagger	4.45 \pm 0.50	5.69 \pm 0.56**	6.59 \pm 0.92** \ddagger

\ddagger p < 0.05 when males were compared with females

** p < 0.001 when IFG and Diabetics were compared with normal individuals

\ddagger p < 0.05 when IFG and diabetics were compared amongst each other

In the various age groups i.e. 18-24 years, >24-30 years and >30-35 years it was observed that levels of fasting plasma glucose increased significantly when Diabetic, IFG and normal individuals were compared with each other. The levels of fasting plasma glucose and Glycated Hemoglobin did not show any significant variation when males and females were compared with each other except for in the age group of 18-24 years, where the levels of fasting plasma glucose were significantly higher in diabetic females as compared to diabetic males (Table 1).

Table 2 Comparison of Total Cholesterol and S. Triglycerides in Normal IFG and Diabetic Individuals belonging to different Age groups

Age Group	Gender	Total cholesterol mg% (Mean \pm S.D)			Triglycerides mg % (Mean \pm S.D)		
		Normal	IFG	diabetic	Normal	IFG	diabetic
18-24 years	Male	183 \pm 3.54	173 \pm 4.0	201 \pm 3.7" ‡	116 \pm 5.43	116 \pm 4.13	191 \pm 4.97" ‡
	Female	185 \pm 3.77	†198 \pm 3.9*	†219 \pm 5.7" ‡	106 \pm 4.48	††159 \pm 8.85**	187 \pm 9.28" ‡
>24-30 years	Male	186 \pm 3.81	191 \pm 4.26	‡215 \pm 4.41**	113 \pm 5.91	143.7 \pm 8.98**	149 \pm 9.70
	Female	189 \pm 3.13	201.8 \pm 3.15	210 \pm 3.57	116 \pm 4.03	155.6 \pm 1.07**	‡†173 \pm 1.38**
>30-35 years	Male	187.2 \pm 3.7	189.3 \pm 4.54	200.1 \pm 5.88‡	153.4 \pm 5.2	157 \pm 8.1	189.9 \pm 9** ‡
	Female	191.9 \pm 4.38	†203.9 \pm 5.1	†214.9 \pm 4.1 ‡	147 \pm 6.2	†177.5 \pm 8.3**	182.3 \pm 1** ‡

† p< 0.05 when males and females were compared amongst each other

** p< 0.001 when IFG and diabetics were compared with normal individuals

*p<0.05 when normal and IFG were compared amongst each other

††p<0.001 when males and females were compared amongst each other

‡ p<0.05 when IFG and diabetics were compared with each other

Levels of Total Cholesterol and S. Triglycerides showed an increasing trend when normal IFG and diabetic individuals were compared amongst each other belonging to various age groups. It was observed that females belonging to the age group of > 18-24 years had higher triglycerides as compared to males belonging to the same age group, whereas the variation in diabetic males and females belonging to age group of >24-30 years showed a significant variation (Table 2).

Table 3 Comparison of Lipoproteins in Normal IFG and Diabetic Individuals belonging to different Age groups

Age Group	Gender	LDL mg% (Mean ±S.D)			VLDL mg % (Mean ±S.D)			HDL mg% (Mean ±S.D)		
		Normal	IFG	diabetic	Normal	IFG	diabetic	Normal	IFG	diabetic
18-24 years	Male	111 ± 3.3	120±3.6	135.64±3.6**‡	18 ±9.94	21±8.5	23 ± 1.08	50.8±8.07	50.0±5.8	47.18±7.
	Female	111 ± 3.4	115±4.3	135.26±4.9**‡	21± 8.94	31±1.79*	‡37±1.85**	51.49±6.2	47.2±7.58	47.0±9.9
>24-30 years	Male	112 ± 4.2	115.6±3.29	133 ±4.61**‡	23 ± 1.21	28.75±1.79	29.94±1.94	50.45±6.6	50.78±7.9	50.90±6.76
	Female	112± 3.6	119.55±3.3	127±4.75**‡	23.55± 8.26	31.12±2.14	‡34.77±2.76*	49.78±7.6	48.40±6.2	49.94±4.11
>30-35 years	Male	109.5±4.2	100.7±4.6	‡115.7 ±5.7	30.4 ±1.6	31.6±1.0	37.9±1.8*	51±6.9	47.4±7.2	45.7±6.2
	Female	115.4 ± 4.4	‡114.7±3.9	‡133.6±4.0**‡	29.6 ± 1.1	35.5±1.6	36.4±2.1	50 ± 7.8	46.6±5.2	46.5±4.5

†p< 0.05 when males were compared with females,

**p< 0.001 when IFG and diabetics were compared with normal individuals,

*p<0.05 when IFG, Diabetics and normal were compared with each other,

‡p<0.05 when IFG and diabetics were compared amongst each other

S. Lipoproteins i.e. LDL, VLDL increased significantly when Normal, IFG and Diabetics were compared amongst each other with an increasing trend in the levels. HDL-C did not vary significantly when Normal, IFG and Diabetic males and females were compared amongst each other (Table 3).

Table 4 Comparison of DNA methylation in Normal IFG and Diabetic Individuals belonging to different Age groups

Age Group	Gender	5mc% (Mean ± S.D.)		
		Normal	IFG	diabetic
18-24 years	Male	2.06±0.36	3.56±0.63*	19.93±8.45*
	Female	2.84± 0.38†	3.63±2.05*	5.09±0.83*††
>24-30 years	Male	3.25±0.29	4.07±0.51*	6.11±2.1*
	Female	2.71±0.58†	4.23±1.3*†	5.24±1.5*†
>30-35 years	Male	1.97±1.3	4.76±0.70*	4.78±3.01*
	Female	2.94±0.69†	3.87±1.3*††	4.56±2.97*†

*p<0.001 when normal and IFG individuals were compared amongst each other

†p<0.05 when males and females were compared with each other

††p<0.001 when males and females were compared with each other

5mc% was more in IFG and Diabetic individuals as compared to normal males and females. The levels were more in Diabetic males as compared to females in the age group of >18-24 years. This difference was statistically significant (p<0.001) Table 4.

Discussion: Diabetes mellitus is a metabolic disorder due to lack of insulin secretion or action or both, resulting in persistent hyperglycemia. Earlier type 2 Diabetes mellitus was considered a disease of middle aged but with prevalent lifestyle changes i.e. increasing obesity and sedentary lifestyle in young individuals the prevalence has increased in adolescents and individuals as young as 18 years of age. As reported in previous studies²⁰ that the age of onset of type 2 Diabetes mellitus is decreasing and there is increased prevalence of insulin resistance in siblings of patients of type 2 diabetes mellitus, the present study focused on family members of Diabetics i.e. off springs of diabetics in the age group of 18-35 years. All the individuals included in the present study were divided into three groups depending on the levels of fasting plasma glucose and Glycated hemoglobin as Normal, IFG and Diabetic and also segregated based on their gender. It was observed that in the age group of 18-24 years the fasting plasma glucose in diabetic females was significantly higher when compared with diabetic males. The females belonging to this age are in their reproductive years and it has been stated that estrogen and sex hormone binding globulin are also associated with insulin resistance in women, thus increased levels of glucose²¹. More the level of glucose more is the glycosylated Hemoglobin.

Dyslipidemia at a young age of >18-24, >24-30 and >30-35 years is indicative of insulin resistance. Levels of triglycerides are more due to increased levels of glucose in circulation although levels of insulin were also more, but the response generated was not significant in lowering the levels of glucose to normal limits in individuals belonging to this age group.

As observed in the present study levels of lipids were more in IFG females belonging to various age groups. Similar observations have been made by OMP Ganda (1985)²². Although in diabetics poor metabolic control is the reason for this change in individuals with IFG this may be

partly explained by the fact that these individuals are still not aware of their status of lipid profile and hence are at an increased risk of developing complications.

Among the most fundamental systems required for survival are metabolic and immune systems and there is strong integration between regulation of metabolism and immune responses. Chronic inflammation due to abnormal production of cytokines and activation of inflammatory signaling pathway is closely associated with metabolic disorders such as insulin resistance²³ and type 2 diabetes mellitus. Inflammatory process plays an important role in pathogenesis of type 2 diabetes mellitus and chronic inflammation precedes the onset of the disease.

DNA methylation is one of the markers of epigenetic variation which has been linked to type 2 diabetes mellitus and its related risk factors including insulin resistance. The genetic basis for developing type 2 diabetes has been recognized for a long time. The concordance of type 2 diabetes mellitus in monozygotic twins is approximately 70% compared with 20-30% in dizygotic twins and a sibling of an affected individual has about 3-time higher risk for developing type 2 diabetes mellitus than the general population²⁴.

The incidence of type 2 diabetes mellitus has increased dramatically over the past decade which is too short a period for consideration of genetic alterations in the genome. Therefore, it is likely that environmental factors including diet and sedentary lifestyle play a significant role in development of type 2 diabetes mellitus.

Epigenetics in the current study was studied by estimating DNA methylation. The levels of 5mc% were increased significantly in individuals with IFG and diabetes (both males and females) especially in the age group of 18-24 years (males). 5mc% indicates the excess of methylation of the genome which is an alarming situation. In individuals with IFG similar

changes were observed which a matter of concern is again because persistent hyperglycemia leads to various complications associated with Diabetes mellitus.

Conclusions:The present study concludes that hyperglycemia, Insulin resistance, altered life style and obesity are risk factors for development of type 2 diabetes mellitus, but these changes are occurring at an early age i.e. 18-24 years (young adolescents) which is a matter of concern. Increased DNA Methylation in these individuals indicates towards deranged metabolism and development of inflammation in these individuals. These changes can be reversed by changing the dietary and lifestyle pattern as Epigenetics is affected by life style.

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