Study of Adiponectin gene polymorphism among off springs of Type 2 Diabetes mellitus.

Dr. Deepa. K*1, Dr. Meera. S 2, Dr. Shubha Jayaram3, Dr. Sunitha DM4, Dr. Savitha Nageshappa5, Dr. Srikanta. BM6, Dr. Sudhir7.

1.Assistant Professor, Department of Biochemistry, Mysore Medical College and Research Institute, Mysore, Karnataka India
2.Professor & Head, Department of Biochemistry Mysore Medical College and Research Institute, Mysore, Karnataka India
3. Professor , Department of Biochemistry Mysore Medical College and Research Institute, Mysore, Karnataka India
4. Professor & Department of Medicine,KR Hospital, Mysore Medical College and Research Institute, Mysore, Karnataka India
5. Dr. Savitha Nageshappa,Research Scientist II,Multidisciplinary Research Unit (MRU), Mysore Medical College and Research Institute, Mysore, Karnataka, India.
6. Dr. Srikanta. BMResearch Scientist I,Multidisciplinary Research Unit (MRU) Mysore Medical College and Research Institute, Mysore, Karnataka India
7. Assistant Professor, Department of Community Medicine, Mandya Institute of Medical Sciences, Mandya, Karnataka, India

Corresponding author: Dr. Deepa. K
Assistant Professor Department of Biochemistry Mysore Medical College & Research Institute
Mysore-560001

Abstract: Adiponectin is exclusively expressed in white adipose tissue. ADIPOQ gene is located on chromosome 3q27 has been identified as T2DM susceptibility locus by genomic studies. Adiponectin modulates a number of metabolic processes including glucose regulation and fatty acid metabolism by exerting antidiabetic, anti-inflammatory and antiatherogenic effects. Many single nucleotide polymorphisms (SNPs) have been detected in ADIPOQ gene, which are associated with a variety of disorders. Mutations in different regions of adiponectin gene have been reported to be associated with obesity, atherosclerosis and type 2 diabetes mellitus. The present study was aimed to find out the distribution of SNP45T>G of Adiponectin gene and its association with offsprings of type 2 diabetes. The healthy volunteers who are aged between 18-22 years of either sex, studying in our institutions namely Govt Medical college, Govt Paramedical and Nursing sciences were selected based on their family history of diabetes, and were assigned into two groups. Group 1(n=150) included those individuals with both parents non diabetic & non hypertensive. Group 2(n=150), included those individuals with one/both parent being type-2 diabetes mellitus. The genotype frequency of SNP45 T>G in exon 2 of adiponectin gene was determined by PCR based restriction enzyme analysis using the restriction enzyme Sma I. (recognition site: CCC;GGG). Three kind of genotypes: wild type TT (470 bp), heterozygous type TG (470 bp, 336 bp, 134 bp) and homozygote mutant type GG (336 bp, 134 bp) were studied. Out of 150 offsprings of type 2 Diabetes parents, 60 had SNP T>G allele and among offsprings of non diabetic parents 25 had T>G allele. On applying Chi square test it was found that there was statistically significant association of T>G allele in cases than in control, the p value was <0.001. Therefore, SNP45T>G in adiponectin gene may be one of the risk factors for type 2 diabetes.

Key word: Adiponectin, Polymorphisms, Diabetes Mellitus, Antiatherogenic, Anti-inflammatory, Fatty acid metabolism

I. Introduction

Diabetes mellitus is a multi factorial, polygenic metabolic disorder which can affect nearly every organ system in the body. However, the mechanisms associated with T2DM remain uncertain. It is widely accepted that T2DM is a complex disease and both environmental and genetic factors can contribute to disease initiation as well as its evolution. According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9
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million by 2025 unless urgent preventive steps are taken [1]. In study by DESIR (Data from an Epidemiological Study on the Insulin Resistance syndrome) a prospective study, 19 common polymorphisms of 14 known candidate genes were analyzed for their contribution to prevalence and incidence of glucose intolerance in middle-aged Caucasian subjects [2]. ADIPOQ is one such gene. The gene is located on chromosome 3q27, a region already known as susceptible to type 2 diabetes and obesity [3]. It spans 17 kb and consists of 3 exons and 2 introns [4]. The gene product is adiponectin which is a protein exclusively secreted from the adipose tissue, mainly the white adipose tissue. Adiponectin has a molecular weight of 30 kDa and composed of 244 amino acids. The protein possesses a short N-terminal variable region followed by collagen repeats and finally a large C-terminal globular domain [5]. It modulates a number of metabolic processes including glucose regulation and fatty acid metabolism by exerting antidiabetic, anti-inflammatory and antiatherogenic effects [6, 7]. Many single nucleotide polymorphisms (SNPs) have been detected in ADIPOQ gene, which are associated with a variety of disorders. Most of the disorders are part of metabolic syndromes e.g. impaired glucose tolerance, obesity, dyslipidemia and type 2 diabetes. Anti-inflammatory and anti-atherogenic properties of adiponectin and the ability to stimulate insulin sensitivity have made adiponectin an important molecule for physiological and pathophysiological studies with the aim of potential therapeutic applications suggesting, a protective role in diabetes development (8). Recent studies have indicated that Hypo adiponectinemia is caused by interactions of genetic factors such as SNPs (Single Nucleotide Polymorphisms) in the adiponectin gene and environmental factors which may be responsible for insulin resistance, type-2 diabetes and metabolic syndrome (9). Studies undertaken on different ethnic groups have shown a strong positive association of the adiponectin gene, SNP45 T>G polymorphism with type 2 Diabetes (10, 11). Hence to summarize, adiponectin plays an important role in glucose metabolism by its favourable effect on insulin-sensitizing action through fatty acid oxidation, increased energy consumption, and stimulation of insulin secretion. There is strong accumulating evidence from several prospective studies that showed low adiponectin levels as a predictor of the onset of Type 2DM. The variations in the genetic factor, termed Single Nucleotide Polymorphisms (SNPs) influencing the phenotype need to be still effectively and comprehensively addressed in Asian-Indian populations. There are no much studies on Adiponectin gene polymorphisms in off springs of type 2 diabetes among south Indian population who are more prone for diabetes. Hence it may help in assessing the early insulin resistance in this population which is responsible for future diabetes & related complications.

II. Objectives

The present study was aimed to find out the distribution of SNP 45T>G of Adiponectin gene (ADIPOQ rs2241766) and its association with offsprings of type 2 diabetes.

III. Material and Methods

It is a Cross sectional study. The healthy volunteers who are aged between 18-22 years of either sex, studying in our institutions namely Govt Medical college, Govt Paramedical and Nursing sciences were selected based on their family history of diabetes, and were assigned into two groups. Group-1(n=150) included those individuals with both parents non diabetic & non hypertensive. Group 2(n=150), included those individuals with one/both parent being type-2 diabetes mellitus. 5ml of fasting venous sample was collected in EDTA tubes. The genotype frequency of SNP45 T>G in exon 2 of adiponectin gene was determined by PCR based restriction enzyme analysis using the restriction enzyme Sma I. (recognition site: CCC; GGG). Three kind of genotypes: wild type TT (470 bp), heterozygous type TG (470 bp, 336 bp, 134 bp) and homozygote mutant type GG (336 bp, 134 bp) were studied

- **DNA sample:** Genomic DNA was isolated from whole blood using Spin column method and stored under -70 C
- **ADIPOQ Primer details:**[13]
  - Human ADIPOQ Forward primer:
  - GAA GTA GAC TCT GCT GAG ATG G
  - Human ADIPOQ Reverse primer:
  - TAT CAG TGT AGG AGG TCT GTG ATG

**PCR Conditions for ADIPOQ gene using PCR master mix from fermentas**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>95 °C</td>
<td>3min</td>
<td>Initial denaturation</td>
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<tr>
<td>95 °C</td>
<td>30sec</td>
<td>35cycles</td>
</tr>
<tr>
<td>55 °C</td>
<td>1min</td>
<td></td>
</tr>
<tr>
<td>72 °C</td>
<td>1min</td>
<td></td>
</tr>
<tr>
<td>72 °C</td>
<td>5min</td>
<td>Final extension</td>
</tr>
</tbody>
</table>

DOI: 10.9790/264X-0504021421 www.iosrjournals.org 15 | Page
Sma I digestion of Adiponectin PCR product:
- 1 µL of Fast Digest Sma I in 1X Fast Digest buffer and 5 µL of DNA (PCR product) in 30 µL of reaction volume were incubated at 37 °C overnight and the whole digestion product was analysed by 1.8% agarose gel electrophoresis.
- 100 DNA ladder was used as a marker.
- The protocols followed in the study were well standardized and performed with strict quality control measures.

IV. Results
The purified PCR products underwent restriction digestion with Sma I. The following fragment sizing patterns were observed by agarose gel electrophoresis:
- a. 372 bp uncut band in homozygous TT
- b. 153 bp and 219 bp bands in homozygous GG
- c. 372 bp, 153 bp and 219 bp bands in heterozygous TG.

Table 1, figure 2 showing the distribution of Polymorphism among offsprings of non diabetic subjects(control) and offsprings of type 2 diabetic subjects (cases) shows, out of 150 offsprings of type 2 Diabetes parents, 60 had heterozygous SNP T>G allele and among 150 offsprings of non diabetic parents, 25 had T>G allele. 90 of cases had homozygous TT & 124 of controls (offsprings of non diabetic subjects). Two of offspring of diabetic subject had homozygous GG & one among offsprings of non diabetic subjects. On applying Chi square test it was found that there was statistically significant association of T>G allele in cases than in control, the p value was <0.001.

Figure 1: Scanned Images of Sma I digest of ADIPOQ gene under UV Transilluminator

Table 1: Showing the distribution of polymorphism among controls & cases

<table>
<thead>
<tr>
<th></th>
<th>TT</th>
<th>T&gt;G</th>
<th>GG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>124 (82.7%)</td>
<td>25 (16.6%)</td>
<td>1 (0.7%)</td>
<td>150 (100%)</td>
</tr>
<tr>
<td>Cases</td>
<td>90 (60.3%)</td>
<td>60 (39.7%)</td>
<td>2 (1.4%)</td>
<td>150 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>117</td>
<td>3</td>
<td>300</td>
</tr>
</tbody>
</table>

Chi-square test was performed to find the statistical significance between the genotypes, p value <0.05
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Result
- Heterozygous T>G: D019, D022, D024, D025, D029, D031, D035, D036, D040, D042.
- Homozygous G>G: D028
- Homozygous T>T: D020, D021, D023, D026, D027, D030, D032, D033, D034, D037, D038, D039, D043 to D056.

Figure 2: Bar diagram of showing the distribution of polymorphism among controls & cases

Table 2, showing the distribution of Polymorphism according to demographic variables among offsprings of non diabetic subjects (control) and offsprings of type 2diabetic subjects (cases) result did not show any statistical significance in demographic variables like sex and body mass index(BMI) p>0.05.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Controls n=150</th>
<th>Cases n=150</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>TT</td>
<td>60</td>
<td>64</td>
<td>44</td>
</tr>
<tr>
<td>TG</td>
<td>10</td>
<td>15</td>
<td>27</td>
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<tr>
<td>BMI&gt;23</td>
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<td>BMI&gt;23</td>
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<tr>
<td>BMI&lt;23</td>
<td>9</td>
<td>16</td>
<td>BMI&lt;23</td>
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</table>

V. Discussion

Adipose tissue stores TGs during energy surplus, but it is also a key endocrine organ secreting various biologically active adipokines involved in the regulation of energy homeostasis. Infiltration of macrophages and lymphocytes in adipose tissue leads to increased production of pro inflammatory adipokines and vasoconstrictors that induce endothelial dysfunction and vascular inflammation (14). By contrast, adiponectin secreted by adipocytes decreased in diabetes and other related pathologies, as local inflammation disrupt adiponectin transcription (15). Activities of adiponectin in glucose and lipid metabolism, vascular endothelial cells, smooth muscle cells, and macrophages (16) confer protection against metabolic syndrome traits. Thus, adiponectin or its signaling pathways are potential therapeutic targets. The serum adiponectin concentration has a strong genetic component, with heritability estimated at 88% (17). Adiponectin has been widely implicated in possessing anti-diabetic properties, besides other documented attributes that include anti-atherogenic and anti-inflammatory properties. Adiponectin gene (ADIPOQ) variability may affect the risk for type 2 diabetes mellitus (T2DM) and complications. An earlier report had implicated genetic variability on adiponectin gene as associated with extracellular levels of inflammatory and angiogenic markers [18]. However, these authors felt that further research is warranted to unequivocally elucidate the role of adiponectin in the development and/or progression of micro vascular disease in T2DM patients. Two major polymorphisms of adiponectin gene, namely SNP + 45 and SNP + 276 have been documented, but with variegated actions governing Insulin sensitivity and IR [19].

The present study showed statistically significant positive association of Adiponectin SNP 45 T>G among offsprings of type 2 diabetic subjects when compared with offsprings of non diabetic subjects with p<0.05. The result of this study corroborates with the findings of several other studies performed on different ethnic groups. This result corroborates with findings of the studies on South Indian population by Devadrita
Biswas et al [11]. Japanese population by Nakatani et al. and Hara et al. [4, 20], on European population by Gable et al. [21], on Spanish population by González-Sáez et al. [22] & on Caucasian population by Menzaghi et al. [23]. In Devadriita Biswas et al. [11] in 2011 showed that the variant forms T>G and G>G are found to be significantly associated with type 2 diabetes mellitus (P<0.01). Subjects carrying the variant types possess almost 4 times greater risk of having type 2 diabetes than those carrying the wild type. Nakatani et al. [4] in 2005 showed association of two single-nucleotide polymorphisms (SNP45T>G, SNP276G>T) with type 2 diabetes in the Japanese population. In their study, SNP45 was associated with insulin sensitivity and obesity (body mass index). Hara et al. [14] in 2002 presented evidence of an association between frequent single nucleotide polymorphisms at positions 45 and 276 in the adiponectin gene and type 2 diabetes (P = 0.003 and P = 0.002, respectively). Subjects with the GG genotype at position 45 or the GG genotype at position 276 had a significantly increased risk of type 2 diabetes. Gable et al. [7] determined the impact of identified adiponectin gene (ADIPOQ) variants 45 T>G (exon 2) and 276 G>T (intron 2) on the prospective risk of coronary artery disease and type 2 diabetes in healthy men. Only the 45T>G variant (3.80) was found to be associated with type 2 diabetes mellitus.

In the light of above data, adiponectin SNP45 T>G polymorphism can be closely correlated with the prevalence of type 2 diabetes mellitus. SNP45 T>G could trigger insulin resistance en route to the development of type 2 diabetes mellitus possibly through changes in mRNA stability, levels of adiponectin and eventually reduced plasma adiponectin concentrations. Genetic predisposition for diabetes may influence adiponectin gene expression leading to decrease in its plasma concentration, which might play a key role in developing diabetes in near future. Impaired adiponectin multimerization due to mutation is usually the causative factor for the disease. Two mutants are incapable of forming the high molecular weight species while three are incapable of forming a stable trimer and show impaired secretion as well [12]. According to studies by Aseel AlSaleh et al. these inconsistencies may relate to the interaction between gene variants and nutrients with potential effects on expression. In particular, heterogeneity of response to fish oil fatty acids within populations has been attributed to genetic variation. Un saturated fatty acids are ligands for the transcription factor PPARγ, which upregulates ADIPOQ gene expression and directly increases serum adiponectin concentration [24].

VI. Conclusion

The study showed statistically significant association of T>G allele in children of type 2 diabetic subjects than in children of non diabetic parents, the p value was <0.05. The study could help in understanding the possible role and influence of adiponectin gene polymorphism particularly SNP45T>G in initiating the lead towards developing diabetes. SNP45T>G in adiponectin gene may be one of the important risk factor for the early insulin resistance and could help in developing personalized medicine and to take preventive measures. The result of this study corroborates with the findings of several other studies performed on different ethnic groups. Therefore, the SNP45 T>G of adiponectin gene may play an important role in the development of type 2 diabetes mellitus. Direct evidence to support SNP45-induced regulation of adiponectin expression is still lacking and warrants further investigation. Direct measurement of adiponectin expression in white adipose tissues obtained from individuals with the SNP45 genotypes and its association with plasma fasting glucose level, insulin level, homeostasis model assessment (HOMA) for insulin resistance can throw more light on the role of SNP45 in the occurrence of type 2 diabetes mellitus.

Acknowledgement

Authors thank the Rajiv Gandhi University of Health sciences, Bangalore, Karnataka for financial support for the project and facility by Multi disciplinary Research Unit, Mysore Medical College & Research Institute, Mysore.

Conflict of Interest: None

References

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p>0.05, not statistically significant.

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This study helped to understand the molecular mechanism involved in Insulin Sensitivity. Therefore, SNP45T>G in adiponectin gene may be one of the risk factors for type 2 diabetes.