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Research Article

The investigation of the role of Neurogenin-3 gene expression and gene polymorphism on the Type 2 Diabetes Mellitus

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Abstract: Diabetes Mellitus (DM) is a chronic metabolic disease causing disorders in carbohydrate, protein and fat metabolism, which occurs as a result of deficiency in the secretion and effect of the insulin hormone. Pancreatic islet cell differentiation and basic helix-loop-helix transcription factor for regeneration neurogenin-3 (Ngn-3) has a critical role. The results obtained with type 2 diabetes susceptibility to the disease, the efficacy of treatment in type 2 diabetics and are intended for receipt of these measures. In this study, the susceptibility to Ngn-3 gene polymorphisms and expression with type 2 diabetes disease has investigated the relationship between treatment and control activities. The materials and methods in this study, Firat University Scientific Research Projects Coordination Unit and ethical approval of the study was performed according to the decision of 11.10.2012 date and No. 01 The present study included volunteered 40 patients who live around Elazığ Province, who were diagnosed with Type 2 DM and who are aged over 30 years (10 male and 10 female who received only oral anti-diabetic treatment and 10 male and 10 female who received no medication) and volunteered 40 individuals in control group (20 male and 20 female) who are aged over 30 years. In addition to routine blood examinations, Neurogenin-3 (Ngn-3) gene polymorphism and gene expression were examined in the study groups. In results the study compared Type 2 DM (n=40) and control (n=40) groups in terms of Ngn-3 rs4536103 gene area polymorphisms. There was no significant difference between these two groups in terms of Ngn-3 area gene polymorphisms rates (p=0,597). Significant correlation was detected between SNP rs4536103of the Ngn-3 expression, polymorphism and BMI (p= 0.04). The conclusion in our study, Ngn-3 Single Nucleotide Polymorphism rs4536103 were no significant differences between regions in terms of type 2 diabetes and control groups. But with the Ngn-3 gene expression Body Mass Index (BMI), a significant relationship was identified between. In addition to the work carried out functional NGN-3 conversion from pancreatic beta cells premise to assume an important role in the future of a new generation of Ngn-3 expression in pathways affecting the pharmaceutical shows they can be used as a target.
Keywords: Type 2 DM, Ngn-3 polymorphism, Ngn-3 expression

INTRODUCTION

Diabetes Mellitus (DM) is a chronically systemic metabolism disease progressing with chronic hyperglycemia. It is characterized by partial or complete deficiency of insulin and / or any disorder in carbohydrate, protein and fat metabolism resulting from insulin resistance [1, 2]. In Turkey, the frequency of diabetes is reported to be 13.7 % according to data from "Turkish Diabetes, Hypertension, Obesity and Endocrine Diseases Prevalence Study II (TURDEP-II Trial)" [3].

In 2010, the American Diabetes Association (ADA) has recommended the use of HbA1c for the diagnosis of DM. The criteria determined for diagnosis of diabetes by the ADA which were revised in 2010 are indicated below [4].

Diagnostic Criteria for Diabetes Mellitus[4]

1. Plasma glucose levels of ≥ 200 mg / dL (≥11.1 mmol / l), together with the symptoms of diabetes, regardless of the time passing after the last meal during the day. (The symptoms for diabetes include polyuria, Polydypsia, and unexplained weight loss) or
2. Fasting plasma glucose levels of ≥126 mg / dl (≥7,0 mmol / l). (Hunger is defined as the time elapsed between 8 hours at least and 14 hours at

most without taking any calories) or

3. Plasma glucose levels of ≥200 mg / dl (≥11,1 mmol / l) at the 2nd hour in the OGTT. (as defined by WHO, OGTT should be performed by using 75 g of glucose dissolved in water at fasting after an intake of sufficient carbohydrates (150 g/day) for 3 days) or
4. HbA1c ≥ 6.5% (this test has been standardized with DCCT (Diabetes Control and Complications Trial) analysis and should be performed in laboratories using a suitable method approved by NGSP (National Glycohemoglobin Standardization Program)).

Diagnosis can be achieved by using one of the above criteria [4].

Studies focus on the presence of pancreatic transcription factor Ngn-3 which is a proendocrine regeneration as well as its importance on new endocrinal cell differentiation in islets. In this context, the position of Ngn-3 in pancreas which is developing and getting regenerated has brought the possible Ngn-3 based therapies for increased endocrinal mass to the subject of debate**.** The basic helix-loop-helix transcription factor Ngn-3 plays a critical role in both pancreatic islet cell differentiation and regeneration. Ngn-3, which arises during primary and secondary endocrinal cell differentiation, allows the formation of alpha, beta, pancreatic polypeptide and gamma cells expressing pancreatic polypeptide, somatostatin, insulin and glucagon. The regulatory role of Ngn-3 in the development of endocrinal pancrease may be important for therapeutic approaches which expand Ngn-3 expression by increasing beta cell masses and their functions [5].

The present study aims at examining Neurogenin-3 (Ngn-3) gene expression and gene polymorphism in diabetic patients.

MATERIALS AND METHOD:

The power analysis performed before the study suggested that at least 11 cases would be required to determine the changes in Neurogenin-3 (Ngn-3) gene polymorphism and gene expression in terms of their role in pathogenesis with a power rate of 90% ($a = 0.05$, $\beta = 0.1$). We thus evaluated the study size in a total of 40 cases in terms of both the correct diagnosis and the reasons that may prevent diagnosis.

This study was performed on a total of 80 subjects who applied to the clinic and polyclinic of general internal medicine in Fırat University, 40 of which were over the age of 30 years who diagnosed with Type II DM for at least 6 months, and the other 40 controls were not diagnosed with diabetes. Participants from both patient and control groups were informed about the study and their written consents were obtained. Those who were diagnosed with Type I DM or below the age of 30 years or using insulin were excluded from the study.

3 mL of peripheral blood was obtained from each group. The collected blood samples were stored at -20 ° C until studied. The materials were subjected to DNA and RNA isolation processes at medical genetics laboratory. Then, Neurogenin-3 gene polymorphism and gene expression were evaluated by means of reverse transcriptase PCR (RT-PCR) method. Those who diagnosed with Type II DM were compared to the controls in terms of polymorphism and expression for each gene. It was also examined the role of neurogenin-3 (Ngn-3) gene polymorphism and gene expression in pathogenesis. SPSS 16 (The Statistical Package for Social Sciences, version 16.0, SPSS Inc, and Chicago, IL, USA) statistical software package was used in the analysis of data sets. T-test and Chi-square tests were used in order to evaluate the numerical data and categorical variables, respectively. $P < 0.05$ was considered statistically significant.

FINDINGS

This study was performed on a total of 80 subjects who applied to the clinic and polyclinic of general internal medicine in Fırat University, 40 of which were over the age of 30 years who diagnosed with Type II DM for at least 6 months, and the other 40 controls were not diagnosed with diabetes.

Polymorphism at Ngn3 rs4536103 gene region

In overall patients with Type 2 DM (n = 40), for Ngn3 rs4536103 gene region, 13 subjects (32.5%) were homozygous (C/C), 16 subjects (40%) were heterozygous (C/T) and 11 subjects (27.5%) were homozygous mutants (T/T). However; in controls without Type 2 DM (n = 40), 9 subjects (22.5%) were homozygous (C/C), 19 subjects (47.5%) were heterozygous (C/T) and 12 subjects (30%) were homozygous mutants (T/T). There is no significant difference between the two groups in genotype of Ngn3 rs4536103 gene region (p: 0.597).

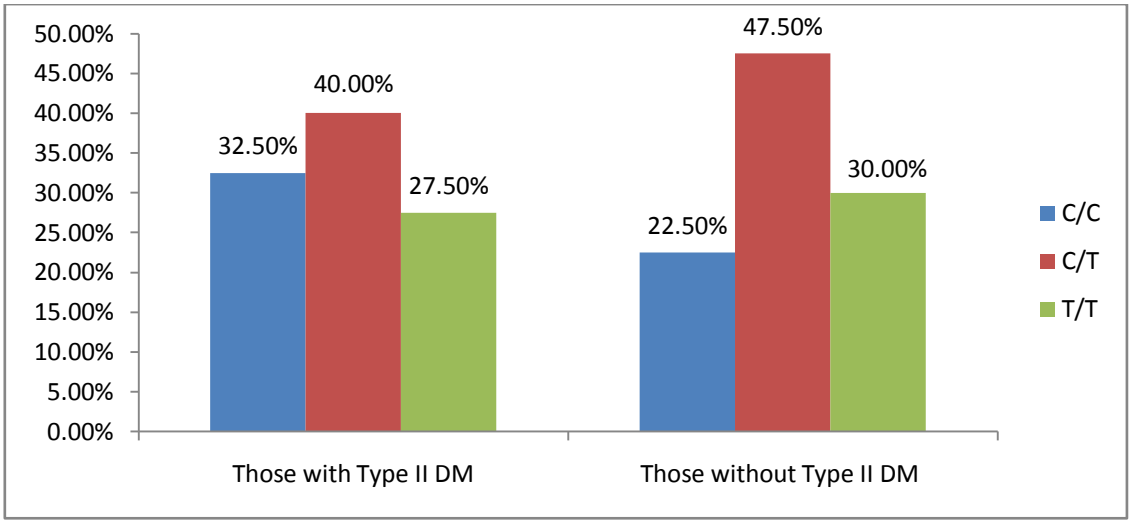

Fig-1: The rates for polymorphism at Ngn3 rs4536103 gene region in patients and controls.

Table 1: Comparison between patient and control group for polymorphism at Ngn3 rs4536103 gene region (X^2 test)

Ngn3 rs4536103	A / A		G/A		G / G		Total	P
	n	%	n	%	n	%		
Those with Type 2 DM	13	32.5	16	40	11	27.5	40 100%	0.597
Those without Type 2 DM	9	22.5	19	47.5	12	30	40 100%	
Total	22		35		23		80	

Ngn-3 rs4536103 gene expression (mRNA level)

When compared to T-test, no significant difference was found between the groups, in terms of Ngn3 rs4536103 gene expression levels in patient and control groups, regardless of polymorphism type in Neurogenin3 rs4536103 gene.

Table 2: The comparison of patient and control groups in terms of Ngn-3 rs4536103 gene expression levels (T-test).

Group	n	Mean	STD Dev.	P
Treated	20	1.8945	2.60123	
Untreated	20	2.3780	3.34345	0.843
Control	40	2.2405	2.45590	

The Comparison of Gene Expression Levels according to Ngn3 rs4536103 gene region in all study groups

In all study groups, when considering how Ngn3 rs4536103 gene expression levels were affected according to C/C, C/T or T/T genotypes, there was no significant difference in expression levels among these three groups (p = 0.471).

Table 3: The Comparison of Expression Levels according to Ngn3 rs4536103 gene region in all study groups (OneWay ANOVA)

	rs4536103	N	Avg. value	Std. deviation	P
Ngn 3 Expression	C / C	22	2.6173	3.06478	
	C / T	35	2.2783	2.83134	0.471
	T / T	23	1.6413	2.10749	
	Total	80	2.1884	2.70662	

Ngn3 rs4536103 gene expression levels - relationship between those with Type II DM treated and untreated

The number of treated subjects was 20, whereas that of untreated subjects was 20. No significant difference was observed in mean expression level of Ngn-3 rs4536103 between those treated and untreated patients as compared to the T-test (p = 0.613).

Table 4: Ngn-3 rs4536103 gene expression levels - relationship between those with Type II DM treated and untreated (T-test).

Ngn3 rs4536103	N	Mean	STD Dev.	p
Treated	20	1.8945	2.60123	
Untreated	20	2.3780	3.34345	0.613
TOTAL	40	2.1362	2.96689	

Ngn-3 rs4536103 gene expression level - relationship between those with and without obese

The number of non-obese subjects was 54, whereas that of obese subjects was 26. There was a significant difference in mean expression level of Ngn-3 rs4536103 between those with and without obesity as compared to the T-test (p=0, 04).

Table 5: Ngn-3 rs4536103 gene expression level - relationship between those with and without obesity (T-test)

Ngn-3 rs4536103	N	Mean	Std. deviation	P
Obese	26	0.9592	1.77345	
Non-obese	54	2.7802	2.88757	0.04
Total	80	1.1884	2.70662	

DISCUSSION

Diabetes Mellitus (DM) is a chronically systemic metabolism disease progressing with hyperglycemia, characterized by partial or complete deficiency of insulin and / or any disorder in carbohydrate, protein and fat metabolism resulting from insulin resistance [1,2].

In Turkey, the frequency of diabetes is reported to be 13.7 % according to data from "Turkish Diabetes, Hypertension, Obesity and Endocrine Diseases Prevalence Study II (TURDEP-II Trial)" [3].

Studies focus on the presence of pancreatic transcription factor Ngn-3 which is a proendocrine regeneration as well as its importance on new endocrinal cell differentiation in islets. In this context, the position of Ngn-3 in pancreas which is developing and getting regenerated has brought the possible Ngn-3 based therapies for increased endocrinal mass to the subject of debate**.** The basic helix-loop-helix transcription factor Ngn-3 plays a critical role in both pancreatic islet cell differentiation and regeneration. Ngn-3, which arises during primary and secondary endocrinal cell differentiation, allows the formation of alpha, beta, pancreatic polypeptide and gamma cells expressing pancreatic polypeptide, somatostatin, insulin and glucagon. The regulatory role of Ngn-3 in the development of endocrinal pancrease may be important for therapeutic approaches which expand Ngn-3 expression by increasing beta cell masses and their functions [5].

In our study, non-obese (n = 54) and obese (n = 26) subjects were compared in terms of Ngn3 rs4536103 gene expression levels. There was a significant difference in mean expression level of Ngn-3 rs4536103 between those with and without obesity as compared to the T-test and Ngn3 expression was found to be associated with BMI (p=0.04)(Table 6).

As a result, there were no significant differences between those with Type II diabetes and control group in terms of Ngn3 rs4536103 SNP region expression and polymorphism, but a correlation between Ngn3 gene expression and BMI was found. Besides functional studies, the induction of pharmacologically and genetically inhibited Ngn3 on insulin resistance suggests that new-generation pharmaceuticals may target the pathways that affect Ngn3 expression [6, 7].

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