Journal of Positive School Psychology
2022, Vol. 6, No. 11, 2111-2117

Comparative Study Of Biochemical Profiles Of Pre-Diabetic, Diabetic And Non-Disease Control In Diabetes Mellitus

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Abstract

T2 diabetes is one of the most emerging issues in the whole World. Ratio of spreading diabetes in Pakistan is also very high. Numerous factors are held responsible for T2 diabetes one of them might be genetic factor. Purpose of this study is to investigate the biochemical signs among diabetics and pre-diabetic control. Populations of the current study were patients registered at DIK and Peshawar district teaching hospitals. Blood samples were taken from the patients after getting their consent. Respondents were categorized into HbA1c and lipid profile. There is a significant mean difference found between body mass index, age and HbA1c among groups. it is concluded that both diabetic and pre-diabetic have abnormal profile and HbA1c. There is no association found due to small sample size. it is recommended for future studies to increase the sample size which might provide interesting results.

Keywords: Biochemical Profiles, Pre-Diabetic, Diabetic, Non- Disease Control & Diabetes Mellitus

INTRODUCTION

Diabetes is one of the issues caused due to abnormal secretion of insulin. If individuals do not have sufficient control on their diet and poor-quality life style would lead to macro and micro vascular diseases. A micro vascular disease creates problems in kidneys, eyes, and nerves. On the other hand pre diabetes is defined as those patients having abnormal pattern of morning sugar, altered level of hemoglobin i.e. 6.0% to 6.4% (Organization, 2000). This disease is very old. As health and medical science was not so advance in the old times. Public do not have sufficient knowledge about these medical issues and their treatment. The current study is one of the pioneer studies to investigate the diabetes and would recommend some curative precautions. People with diabetes issue would feel frequent urination, and thirst (Ebers Payrus, 1500 BC). A theory on diabetes given by Ebers was challenged and rejected by Egyptian endocrinologist having strong control and grip on knowledge of medicine (Ebers papyrus paul Ghalioungui, 1908-1987). He proposed "treatment of thirsty woman (Ghalioungui, 1987). It is assumed that old time Egypt are not able identify the symptoms. Later on an Indian surgeon Sushruta conducted lot of study on diabetes around fifth century and named it madhumeha (urine like honey). She invented sweet urine taste which is sticky and stimulus for ant due to sweet nature. She also found that this disease is found in upper class and it has relation with sugary foods, rice, sweets and cereals (Peumery, 1987). Another Chinese researchers studies diabetes and called it toas means the Chinese Hippocrates in which one person has increase urination, desire to eat more and weight

loss (ca. 160-ca.2019). in seventh century Chinese called it ping Hsiaikho it means extensive drinking of water, urination, desire to eat sugary foods (Peumery, 1987). Further study on diabetes brings interesting results such as infection in skin, ulcers, eye sight abnormalities. In eleventh century Muslims scientists celebrate Arabo Islamic physicians in which scientists named Avicenna (980-1037) wrote a book (El-Kanun) canon of medicine in which he explained that major complications would occur like gangrene and sex abnormalities. More study was conducted by Medie val scholar MOises Maimonides (1138-1204) (Peumery, 1987; (Karamanou, Protogerou, Tsoucalas,Androutsos,&Poulakou-Rebelakou,2016).

Problem Statement

In Pakistan ratio of diabetic patients is increasing rapidly and it is crucial to find out the root cause of the diabetes.

Objectives& Hypothesis

1. FTO is significantly associated with diabetes mellitus in particular context.

MATERIALS AND METHODS

Operative Descriptions

Tool used to find out the location of disease gene is genetic linkage analysis. Another approach to further confirm the linkage of disease called candidate gene was used. Fat mass and obesity gene (FTO) was also used. dioxygenase, gene expression, allele-specific PCR (AS_PCR), hardy-Weinberg law was used.

Sample Size Calculation

Three hundred patients were selected for the current study on the basis of HbA1c.

Table 1: HbA1c categories

Groups	Description	HbAlc
1	Non-diabetic Controls	< 6 %
2	Pre-diabetics	6-6.4 %
3	Diabetics	> 6.5%

Sampling Technique

Non-probability purposive sampling technique was used to register the respondents. Patients included in this study were based on confirm diagnosis of Type two diabetes mellitus. Those below age of 20 years and above 60 were excluded.

Sample Collection

Samples of blood from the patients were divided into coated gel tubes and EDTA tubes and later on centrifuged at 4000 rpm to collect serum. EDTA tubes were freeze at minus 20 degrees centigrade to extract DNA and genetic analysis. Solution used in laboratory experiments

Table 2: Solution used in DNA extraction

S NO	NAME OF SOULTION	STRENGHT	QUANTITY
1	Ethylenediaminetetraacetic acid (EDTA)	0.5 M	500mL
2	Magnesium chloride (Mgcl2) solution	1 M	100 mL
3	Sucrose solution	320mM	500mL
4	Triton X 100	1%	50mL
5	Sodium Dodecyl Sulfate (SDS) solution	2%	100mL
6	Ammonium Acetate Solution	0.5M	100mL
7	Saturated Sodium Chloride (NaC1) solution	5M	200mL
8	Tris-hcl solution	01 M	1000mL
9	Cell lysis buffer	-	1000mL
10	Nucleic lysis buffer	-	1000mL
11	Tris-EDTA (TE) buffer	-	500mL
12	Chloroform	100%	-
13	Ethanol	100%	-

Table 3 FTO- rs9939609 primers sequence

Common Forward		Major Allele Reverse	Minor Allele Reverse
CTATCCAAGTGC ATCAGT		CTATCCAAGTG CATCAGT	CTATCCAAGT GCATCAGA
Product size	-	172	173

Table 4 Reaction mixture of FTO-rs9940128 genotyping

Reagents	Quantity
Deionized water	13 µl
10x PCR Buffer	2.0 µl
50mM MgCL2	0.8 µl
10mM dNTPs	1.0 µl
Primer Forward (F)	0.4 µl
Primer Reverse (R)	0.4 µl
5u/ulTaq DNA Polymerase	0.4 µl
DNA Template	2.0 µl
Total	20µl

Table 5 PCR amplification profile

	Temperature (oC)	Duration (minutes)	No of Cycles
Activation	95	3	1
Denaturation	94	1.0	
Annealing		.40	30
Extension	72	1.0	
Final Extension	7	10.0	1
Soak	4	8	Hold

Table 6 Subjects' Demographic and Anthropometric Data

Characteristics	Nondiabetic controls	Prediabetics	Diabetics	p-value
Total Number	100	100	100	-
Gender (M/F)	63/37	48/52	54/46	0.1
Age (years)	47.23±8.48	40.19±8.44	56.29±8.66	< 0.0001***
BMI	20.41±4.6	25.7±4.1	30±6	< 0.0001***

Table 7 HbA1c (Mean ± Standard Deviation)

Nondiabetic controls	Prediabetics	Diabetics	ANOVA
5.505±0.32	6.22±0.15	9.2±2.2	P < 0.0001

Table8 Lipid Profile

	Non-Diabetics	Pre-diabetics	Diabetics	One-way Anova (p- value)
Total Cholesterol	185.64±19.3	185±31	201±26.5	< 0.0001***
HDL	54±13.5	47±14	57.9±18.1	< 0.0001***
LDL	60±14.5	, 90± 31.7	74±18	< 0.0001***
TG	88±32	128±41	88±34	< 0.0001***
VLDL	43±22	20.2±7.5	21±16 5	< 0.0001***

Figure 1 Cholesterol Level Analysis

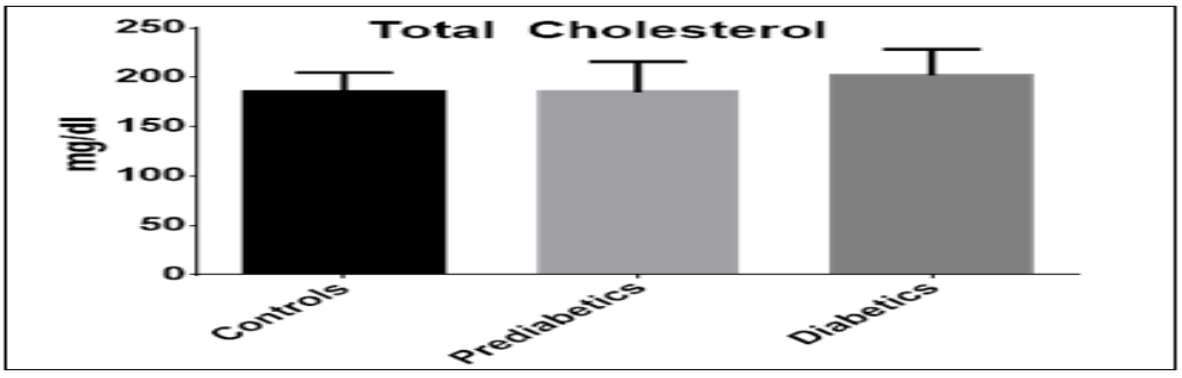


Figure 2 Total Cholesterol in g/dL.

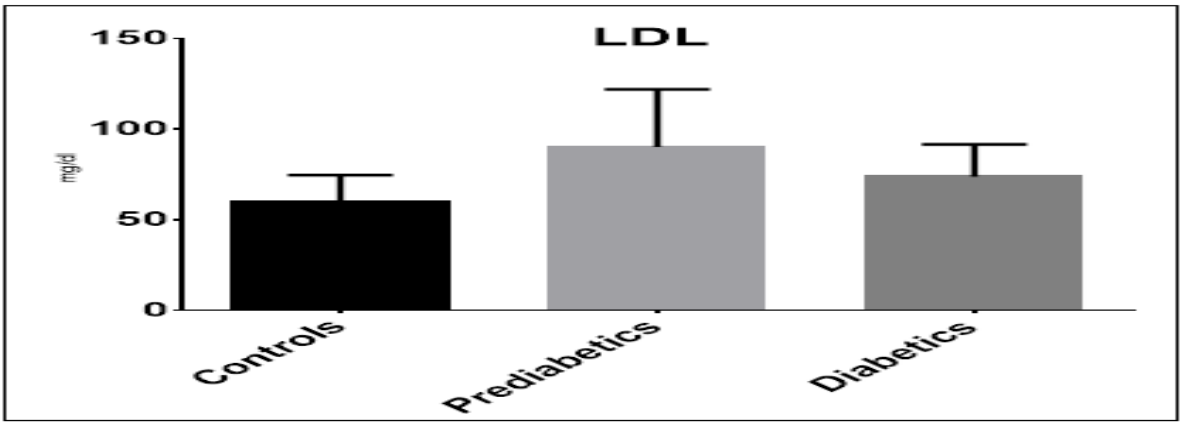


Figure 3 LDL Cholesterol levels among the groups

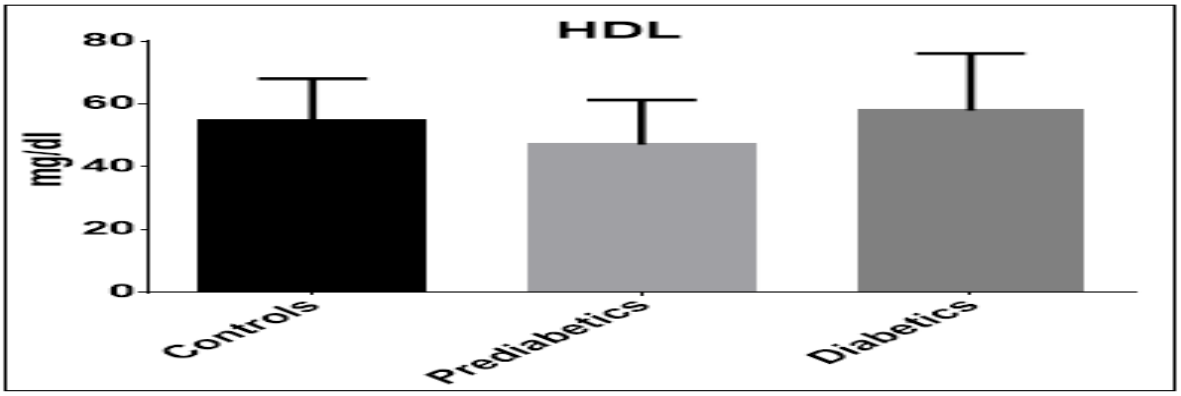


Figure 4 Triglycerides assay results among the groups.

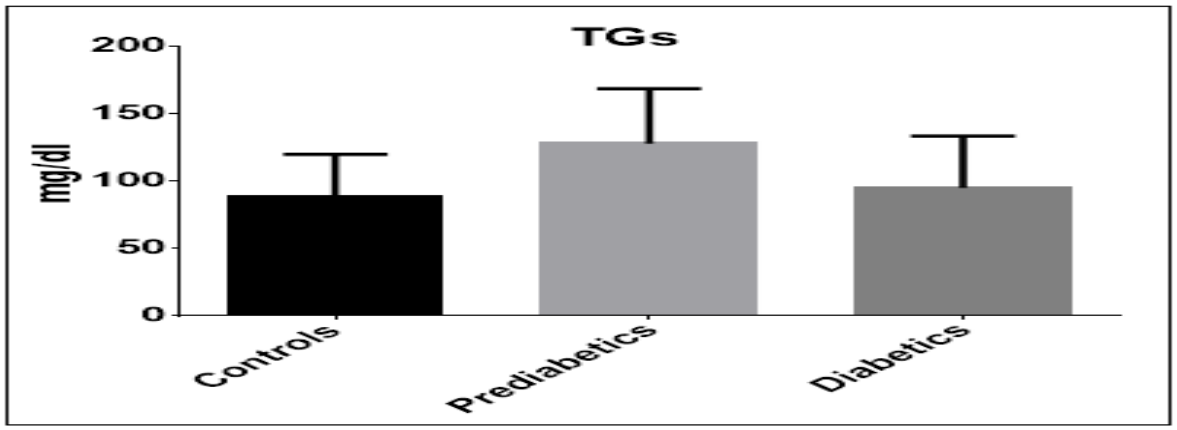


Figure 5 Extracted DNA resolved on 0.7% Horizontal Agarose gel

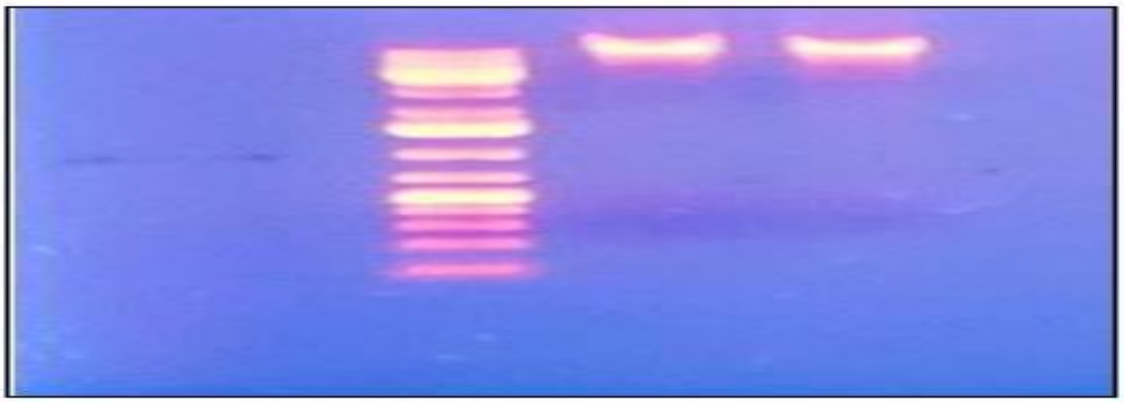
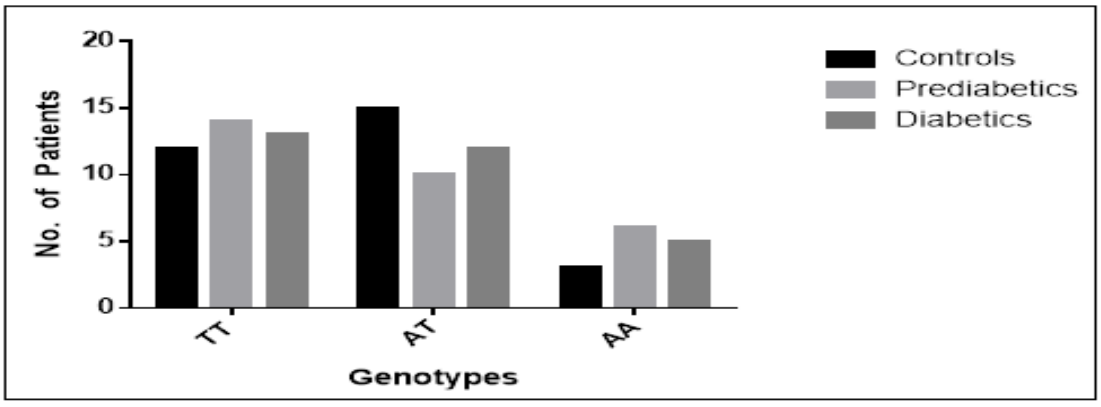


Table 9 Genotype Frequencies among Patients

Genotypes	Controls	Pre-diabetics	Diabetics	χ2 (p-value)
TT	12	14	13	
AT	15	10	12	0.702
AA	3	6	5	

Figure 6 Distribution of FTO genotypes among Controls, Prediabetics and Diabetics.



CONCLUSION

Diabetes is one of the byproduct of industrial revolution. Inactive life style and access to food is easy made the people more diabetic. Type 2 diabetes mellitus is due to uncontrolled glucose in the blood and deregulation of insulin release. Diabetes has several damaging effects such as high level of sugar in blood, damage to organs of the body. It also has effects on life expectancy obesity and life style.

Limitations and Future Work

One should be careful while generalizing the results to other cities because change in demographics, lifestyle, location, brought different findings in other locations. Sample size is small therefore it is recommended future studies may use big sample size to have better understanding of the subject matter.

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