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Original Research Article

Molecular Study on Single Nucleotide Polymorphism of Adiponectin Gene and its Association with Type II Diabetes in Egyptian Patients

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Abstract

Background: Type 2 diabetes mellitus is a prevalent hereditary condition that has multiple genetic risk factors as well as environmental influences. ***Aim*:** The Work aimed to find if there is an association between the presence of Single Nucleotide Polymorphism of the Adiponectin gene & its association with type II diabetes in Egyptian patients. ***Material & Techniques*:** This case-control research had been conducted at Molecular Biology and Biochemistry, Genetic Engineering & biotechnology institute, university of Sadat city. This research had been conducted on eighty patients; separated into 2 groups diabetic group and the control group. ***Results*:** significant reduction in adiponectin level in type II diabetes studied cases compared to normal control subjects ($p < 0.001$). GG genotype is significantly more predominant in type 2 diabetes (54%) while the GT genotype is more predominant in control (60%) (p-value =0.04)., while no variation in allele distribution among both groups (p value=0.6). ***Conclusion*:** In studied cases with type 2 diabetes, there was a significant reduction in adiponectin levels in type II diabetes patients. GG genotype is significantly more predominant in type 2 diabetes. The genotype GG significantly rises the risk of occurrence of type 2 diabetes.

Keywords: Single nucleotide polymorphism, adiponectin gene and type II diabetes.

INTRODUCTION

Type 2 diabetes mellitus is a prevalent hereditary condition that has multiple genetic risk factors as well as environmental influences. It is a chronic condition that necessitates ongoing medical care to prevent the onset of its life-threatening problems & to treat them once they do arise (Ooda *et al*., 2016).

Majority of these problems pose risk to life. elevated death rates linked to DM are partially explained by the emergence of these comorbidities. For instance, diabetes mellitus is linked to a higher risk of hypertension, atherosclerosis, & renal failure (Howlader *et al*., 2021). According to International Diabetes Federation, DM and its consequences caused Five million fatalities in the only year 2015 alone (Zhou *et al*, 2016).

Adiponectin is a protein that adipose cells express & produce. the potential of adiponectin activity to improve insulin action in its target tissues is 1 of several metabolic benefits associated with it (Alfaqih *et al*., 2022).

Different patterns of adipose tissue distribution may account for individual variances in serum adiponectin levels. Yet, 1 genetic investigation discovered that hereditary additive impacts may account for eighty percent of the variation in serum adiponectin levels between nonobese participants (Fadel *et al*., 2020).

SNP in the ADIPOQ gene known as Rs266729 is thought to control promoter activity. The g allele of rs266729 had been discovered to be connected to lower levels of serum adiponectin & higher risk of

hyperglycemia in research that included 1004 adult obese subjects (Truong *et al*., 2016).

Another SNP in the ADIPOQ gene related to variations in serum adiponectin is rs1501299 which is commonly associated with T2DM, IR, and obesity (Palit *et al*., 2020).

The Work aimed to find if there is an association between the presence of Single Nucleotide Polymorphism of the Adiponectin gene & its association with type II diabetes in Egyptian patients.

MATERIALS AND METHODS

This case-control research had been conducted at Molecular Biology and Biochemistry, Genetic Engineering & biotechnology institute, university of Sadat city. This research had been conducted on eighty patients; separated into 2 groups' a diabetic group and a control group.

Inclusion criteria: Patients diagnosed based on their fasting blood glucose depend on American Diabetes Association criteria, (American Diabetes Association; 2008), Patients in the control group had fasting plasma glucose levels under one hundred mg/dl & no history of glucose intolerance. Patients in the study group were ≥fifty years old, taking anti-diabetic drugs, & had no first-degree relatives with T2D.

Exclusion criteria: Individuals using any type of insulin who refuse informed consent & who have any electronic records referencing the existence of any of the following diabetes problems: retinopathy, neuropathy, nephropathy, or atherosclerosis.

Eligible subjects involved in this research had been subjected to the following:
Informed consent had been obtained from each participant.

Demographic: Age, gender, duration of disease, medications, and Co-morbidities (hypertension, cardiac, hepatic, or renal pathology).

Full physical examination: Vital signs including pulse and blood pressure and anthropometric evaluation included weight in kilograms & height in centimeters as follows:

Blood sampling: After an overnight fast, blood samples from each patient had been taken & separated into 3 groups: For genomic DNA extraction & HbA1c, one ml of whole blood had been collected into tubes containing EDTA; for fasting blood glucose, one ml of whole blood had been taken into tubes containing fluoride.

Biochemical measurements: glucose oxidase method had been used to measure fasting blood glucose levels (Spinreact, Girona, Spain). Routine enzymatic techniques had been used to test the levels of total cholesterol & triglycerides (Spinreact, Girona, Spain). Once apoB-containing lipoproteins were precipitated, HDL cholesterol concentration had been measured. Friedewald formula had been used to determine LDL cholesterol levels. We used the Jaffe reaction method to determine the serum creatinine concentrations (Spinreact, Girona, Spain). Using a turbidimetric approach, albumin levels in 24-hour urine samples were measured (Stanbio Laboratory Inc., San Antonio, TX, USA). Based on the manufacturer's instructions, quantities of adiponectin in serum had been found using a double antibody sandwich ELISA (kit supplied by Biosource Europe S.A., Belgium).

DNA extraction: Following the manufacturer's instructions, genomic DNA had been extracted from a second venous blood sample of five ml (in EDTA tubes) using QIAamp DNA Mini Kit. -eighty °C had been used to preserve isolated DNA for SNP genotyping.

Adiponectin gene polymorphisms analyses: Using the Tetra ARMS-PCR technique, adiponectin gene polymorphisms (rs266729 & rs1501299) had been assessed. This technique is quick, sensitive, & easy to use while researching SNPs. After obtaining the genomic DNA sequence for adiponectin (NT_005612.16) from the NCBI website, outer & inner primers had been created.

Administrative and Ethical Design: Official permission was obtained from Molecular Biology and Biochemistry, Genetic Engineering & biotechnology institute, university of Sadat city. Approval from the ethical committee in the institute (Institutional Research Board IRB).

Statistical Analysis: statistical software SPSS version 26 was used (SPSS Inc., Chicago, USA). Using HWE CALCULATOR, SNP's Hardy-Weinberg equilibrium was evaluated (Sharma R *et al*., 2020). chi-square test was used to evaluate genotype distribution & allelic frequencies between people with type II diabetes & controls. Logistic regression analysis was used to determine odds ratios & ninety-five percent confidence intervals to survey the relationship among genotypes & T2D. a p-value of less than 0.05 had been regarded as significant when comparing numerical data that had been expressed as mean ±SD & compared using an independent t-test.

RESULTS

Internal medicine department of Cairo University's College of Medicine provided participants for the current research, which involved 100 participants who had been of similar years old & gender.

The subjects had been separated into 2 groups: Group(I): fifty healthy volunteers as control subjects. **Group(II):** fifty patients with type II diabetes

Table (1) shows a comparison among mean values ± SD of some demographic & biochemical data between studied groups. No variation in gender distribution among studied groups (p-value =0.8).

Table (2) and figure (8) show a rise in insulin levels in type II diabetes studied cases compared to normal control subjects (p = 0.008).

Table (3) and figure (9) show a reduction in adiponectin levels in type II diabetes studied cases compared to normal control subjects (p <0.001).

Table (4) showed that the GG genotype is significantly more predominant in type 2 diabetes (54%) while the GT genotype is more predominant in control (60%)(p-value =0.04)., while no variation in allele distribution between both groups (p value=0.6).

Table (5) the genotype GG significantly increases the risk of occurrence of type 2diabetes (p value <0.001) odd ratio 2.235(1-5.06).

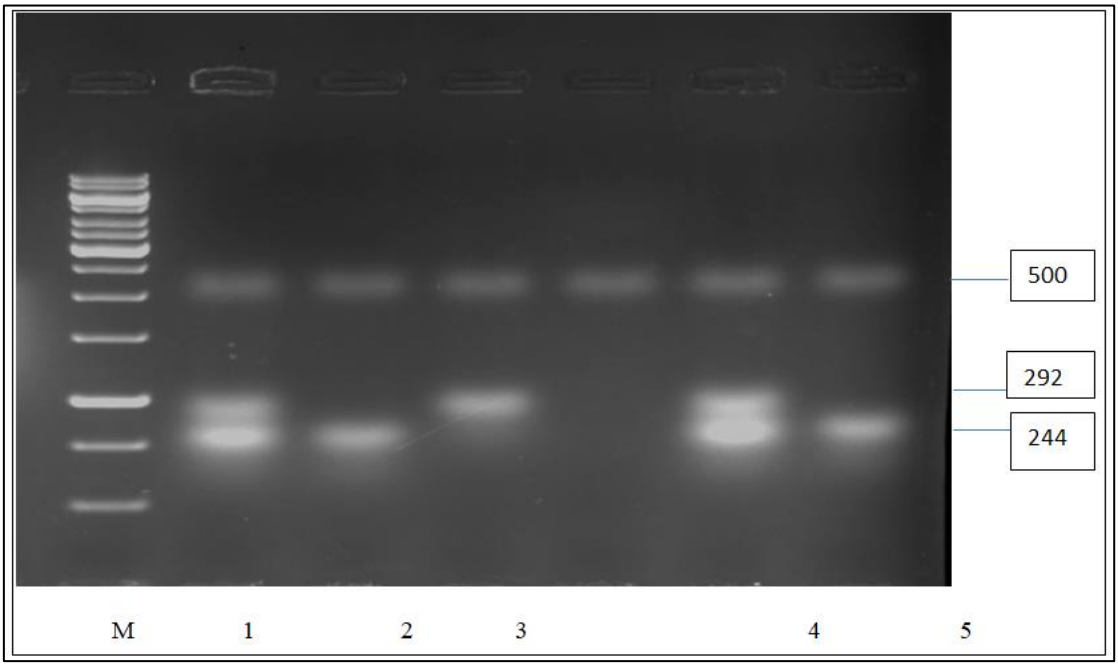

Figure 1: AGAROSE GEL ELECTROPHORESIS SHOW PCR PRODUCT IF …….M DNA LADDER WITH 100BP; LANE 1,4 GENOTYPING GT (G 292 BP) AND (T 244 BP) AND INTERNAL CONTROL(500BP), LANE 2,5 GENOTYPING TT AND (T 244 BP) AND INTERNAL CONTROL(500BP), LANE 3 GENOTYPING GG (G244 BP) AND INTERNAL CONTROL(500BP)

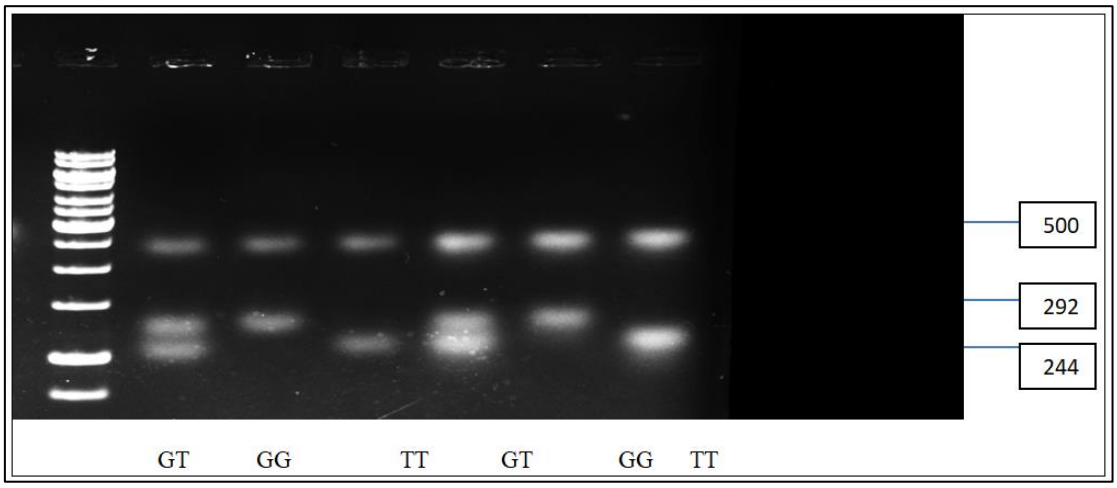

Figure 2: PCR electrophoresis

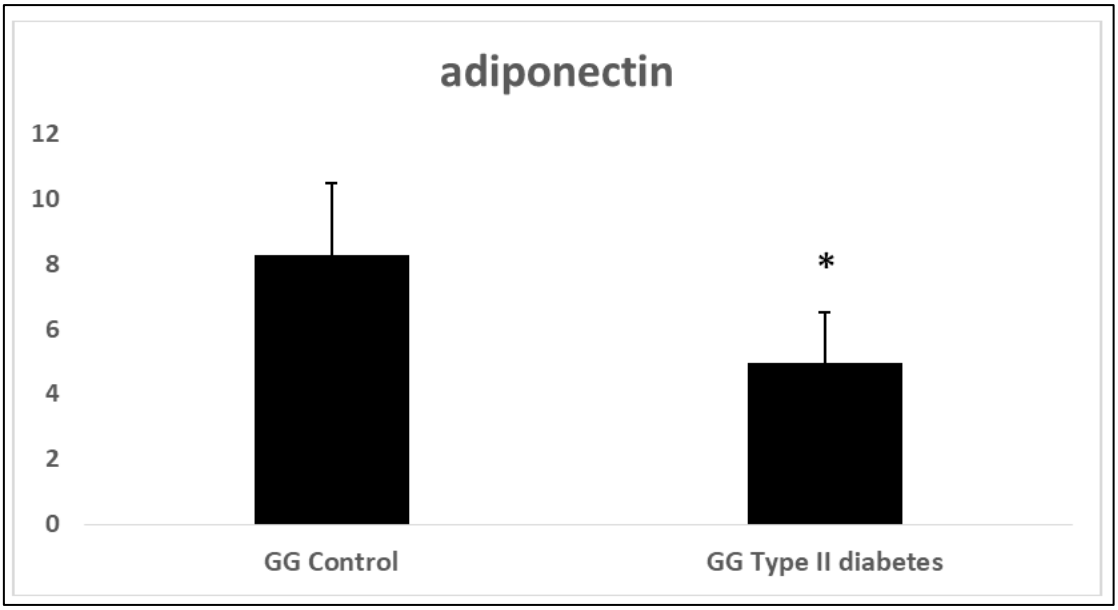

Figure 3: Adiponectin

STATISTICAL SIGNIFICANT decrease in adiponectin in GG genotype of diabetic compared to GG OF CONTROL (P value <0.001)

Table 1: Demographic & biochemical laboratory data between studied groups

Variable	Control Group(I) n=fifty	Type II diabetes Group(II) n=fifty	P1 value*
Sex **Females: n (%)** **Males: n (%)**	 35 (70%) 15 (30%)	 34 (68%) 16(32%)	0.8
Age (years)	45.7±13.4	48.9±8.7	0.76
FBS (mg/dl)	87 +10.7	165.8+63*	< 0.001
2PP	111.33+12.5	296.45+11.5*	<0.001
Cholesterol	187.8 + 34.3	198.6+32.22	0.1
TG	148.9 + 43.7	243.5 + 171*	<0.001
HDL	49.6+7.4	42.7+13.07*	0.002
HbA1c	5.25+0.5	8.94+2.25*	0.019
BMI	29.8+1.14	30.48+10.86	0.8
HOMA IR	1.47+0.9	4.3+3.06	<0.001
HOMA B	157+153	51.68+45.98	<0.001

Data had been expressed as Mean ± SD, p value<0.05 had been significant
(*) Denotes significant variation versus control subjects
FBS: fasting blood sugar, **HbA1c**: hemoglobin A1c, **BMI**: body mass index.

Table 2: Serum insulin by ELISA Technique among Studied Groups

	Control Group(I) n=fifty	Type II diabetes Group(II) n=fifty	P value*
Insulin	7.6+4.8	17.13+32.74*	0.008

Table 3: Adiponectin level by ELISA Technique among Studied Groups

	Control Group(I) n=fifty	Type II diabetes Group(II) n=fifty	P value
Adiponectin	8.28+2.17	4.49+1.96*	<0.001

Table 4: RS1501299 genotype and Allele distribution among studied groups

		Groups		
		control	Type II diabetes	p-value
RS1501299	GG	20(40%)	27(54%)	0.04
	GT	30(60%)	20(40%)	
	TT	0(0%)	3(6%)	
Allele	G	70(70%)	74(74%)	0.6
	T	30(30%)	26(26%)	

Table 5: Regression analysis of RS1501299 genotype distribution among studied groups

		control	Type II diabetes	P value	Odd ratio (95% CI)
RS1501299	GT	30(60%)	20(40%)		
	GG	20(40%)	27(54%)	<0.001	2.235(1-5.06)
	TT	0(0%)	3(6%)		4.56(4.56-4.56)
Allele	G	70(70%)	74(74%)	0.5	0.8(0.4-1.5)
	T	30(30%)	26(26%)		

The genotype GG significantly increases the risk of occurrence of type 2diabetes (p value <0.001) odd ratio 2.235(1-5.06).

DISCUSSION

Adiponectin may protect the cardiovascular system by preventing inflammatory agents from damaging blood vessels, particularly vascular endothelial cells (Sharma *et al*., 2020).

Epidemiological studies have extensively examined 2 polymorphisms, such as silent C to G change in the proximal promoter (rs266729) & silent G to T mutation in intron 2 (rs1501299). Several investigations have revealed that T2DM, IR, & obesity are frequently linked to variant alleles at the rs266729 & rs1501299 polymorphisms (Alimi *et al*., 2021).

This research evaluated many areas of the adiponectin (ADIPOQ) gene that has been linked to type 2 diabetes. It is a case-control investigation. 100 volunteers in all, comprising 50 T2D studied cases & 50 healthy controls, ranging in age from 28 to 65. All participants were subjected to biochemical analysis and Tetra Armer PCR. In our research, there had been no variation in sex distribution among the studied groups.

Our findings agreed with (Alimi *et al*., 2021) who conducted a case study with control. A total of 210 participants had been chosen from the Iranian community, containing 110 healthy controls & 100 T2D sufferers. Using the Tetra ARMS-PCR technique, polymorphisms SNP-11377 C > G & SNP + 276 G > T had been examined. among control & case groups, there had been not any discernible variations in years old or gender.

Similarly, (Wang *et al*., 2018) examined the relationship between adiponectin & type 2 diabetes in 571 age- & sex-matched controls & T2D cases in Singapore Chinese Health Study. Their results found the mean age of participants had been 59.7 (6.2) years & 58.7 percent had been females in the two groups.

Moreover, (Khan *et al*., 2017) conducted on 300 participants, aged twenty-five to seventy-five, including 150 T2DM patients & 150 healthy controls. Blood sugar levels during fasting & after meals, lipid profiles, & serum creatinine were all examined biochemically. years old & sex among case & control groups had been reported to be similar (p>0.05).

In the current study, there had been a rise in FBS and 2PP in type II diabetes studied cases compared to normal control subjects (p <0.001).

Consistent with our results, (Alimi *et al*., 2021) concluded that the FBS was different among T2D studied cases & control subjects (P < 0.05) as increased in T2D patients than the controls.

In our study, there was a rise in HBA1c in type II diabetes studied cases compared to normal control subjects (p =0.019). In agreement with our outcomes, (Wang *et al*., 2018) showed cases had higher levels of HbA1c than controls (P <0.001).

This is in line with (Khan *et al*., 2017) who documented that HbA1c was increased in T2DM cases as compared to healthy controls (p<0.001).

In the present study, there was no variation in cholesterol levels between type II diabetes studied cases & normal control subjects (p =0.1).

In consistency with our study, (Shokri and Golmohammadi; 2014) mentioned that there had been no discernible variation between the 2 groups' cholesterol levels, according to comparing the 3 groups' data (P<0.71).

In the current study, there was a rise in insulin levels in type II diabetes studied cases compared to normal control subjects (p = 0.008).

However, (Alimi *et al*., 2021) documented that insulin levels in type II diabetes patients had been lower compared to controls (P=0.003).

Also, (Kamenova 2006) included twelve patients' body mass who received oral alpha-lipoic acid treatment for four weeks at a dose of 600 mg twice daily. In terms of insulin sensitivity, 12 individuals with normal glucose tolerance served as the control group(Is). They demonstrated that steady-state plasma insulin levels among DM2 studied cases before & after therapy & control group did not differ in a meaningful way.

Our study showed that there was a reduction in adiponectin levels in type II diabetes studied cases compared to normal control subjects(p <0.001).

In agreement with our study, (Tuppad *et al*., 2022) evaluated and associated the glycemic status of studied cases with T2DM & healthy controls with serum levels of adiponectin & nitric oxide. research comprised 50 studied cases with T2DM & 50 studied cases without T2DM who had been between ages of thirty & sixty. Type 2 diabetic studied cases had been categorized into group I. Healthy controls had been considered group II. They reported that the adiponectin was lower in group I compared to groupII (P<0.001).

In the same line, (Kocot *et al*., 2017) Leptin, adiponectin, resistin, & visfatin concentrations were examined, as well as the leptin/adiponectin ratio in plasma of type 2 diabetes studied cases about the degree of obesity. Twenty healthy individuals & ninety-two T2D subjects were included in the research. subjects were classified into four groups based on their BMI values: I(normal body weight), II(overweight), III(obesity), & IV(severe obesity) (control group).

The present research results showed that the GG genotype is significantly more predominant in type 2diabetes (54%) while the GT genotype is more predominant in control (60%) (p-value =0.04)., while no variation in allele distribution among both groups (p value=0.6).

In disagreement with our study, (Karimi *et al*., 2018) included eighty people in the diabetic-prediabetic group who had fasting plasma glucose >one hundred (mg/dl) & eighty people in the control group who had fasting plasma glucose between seventy & one hundred (mg/dl). They discovered that more control cases have GG genotypes (P< 0.001).

According to our study, the genotype GG significantly rises the risk of occurrence of type 2 diabetes (p-value <0.001) odd ratio 2.235(1-5.06).

In agreement with our study, (Alimi *et al*., 2021) documented those frequencies of genotypes of GG (OR = 2.43, P = 0.031), in SNP-11377 C > G polymorphism had been related to a rise in the risk of T2D compared to the control group.

Additionally, (Khan *et al*., 2017) It has been noted that in T2DM cases, the GG genotype of rs266729 significantly affects the levels of circulating adiponectin. Female GG carriers are twice as likely to develop diabetes as men. No significant difference in adiponectin levels between different genotypes (p-value =0.18).

In consistency with our study, (Karimi *et al*., 2018) noted that In both SNPs, levels of adiponectin in diabetic subjects were identical to those in the control group.

CONCLUSION

In studied cases with type 2 diabetes, there had been a significant decrease in adiponectin levels in type II diabetes patients. GG genotype is significantly more predominant in type 2 diabetes. The genotype GG significantly rises the risk of occurrence of type 2 diabetes.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable.

Availability of data and materials: All data and materials are fully presented in the manuscript.

Competing Interests: The authors declare that they have no competing interests.

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REFERENCES

- Ooda, S. A., El-Belbesy, M. F., Hassanein, N. M., Elgaddar, O. H., & Bachlah, H. M. (2016). Assessment of the association of the adiponectin gene single-nucleotide polymorphism 45T/G with type 2 diabetes mellitus in Egyptian diabetic patients. *Egyptian Journal of Obesity, Diabetes and Endocrinology*, *2*(1), 23.
- Howlader, M., Sultana, M. I., Akter, F., & Hossain, M. M. (2021). Adiponectin gene polymorphisms associated with diabetes mellitus: A descriptive review. *Heliyon*, *7*(8), e07851.

- Zhou, B., Lu, Y., Hajifathalian, K., Bentham, J., Di Cesare, M., Danaei, G., ... & Gaciong, Z. (2016). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4· 4 million participants. *The lancet*, *387*(10027), 1513-1530.
- Alfaqih, M. A., Al-Hawamdeh, A., Amarin, Z. O., Khader, Y. S., Mhedat, K., & Allouh, M. Z. (2022). Single nucleotide polymorphism in the ADIPOQ gene modifies adiponectin levels and glycemic control in type two diabetes mellitus patients. *BioMed Research International*, 6632442.
- Fadel, H. H., Hefny, N. E., Yousef, A. I., Sadek, N. A., Attea, E., & El-Sewedy, T. (2020). The association of adiponectin polymorphism (rs2241766) with susceptibility and prognosis of Egyptian patients with hematological malignancies: A case-control study. *International Journal of Cancer and Biomedical Research*, 4(1), 57-68.
- Truong, S., Tran, N. Q., Ma, P. T., Hoang, C. K., Le, B. H., Dinh, T., ... & Do, M. D. (2022). Association of ADIPOQ single-nucleotide polymorphisms with the two clinical phenotypes type 2 diabetes mellitus and metabolic syndrome in a Kinh Vietnamese population. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 307-319.
- Palit, S. P., Patel, R., Jadeja, S. D., Rathwa, N., Mahajan, A., Ramachandran, A. V., ... & Begum, R. (2020). A genetic analysis identifies a haplotype at adiponectin locus: association with obesity and type 2 diabetes. *Scientific reports*, *10*(1), 2904.
- American Diabetes Association. (2008). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 31, 55-60.
- Sharma, R., Satyanarayana, P., Anand, P., & Kumari, G. A. (2020). Adiponectin level association with MDA in the patients with type 2 diabetes mellitus. *Biomedical and Pharmacology Journal*, *13*(2), 943-955.
- Alimi, M., Goodarzi, M. T., & Nekoei, M. (2021). Association of ADIPOQ rs266729 and rs1501299 gene polymorphisms and circulating adiponectin level with the risk of type 2 diabetes in a population of Iran: a case-control study. *Journal of Diabetes & Metabolic Disorders*, *20*, 87-93.
- Wang, Y., Meng, R. W., Kunutsor, S. K., Chowdhury, R., Yuan, J. M., Koh, W. P., & Pan, A. (2018). Plasma adiponectin levels and type 2 diabetes risk: a nested case-control study in a Chinese population and an updated meta-analysis. *Scientific reports*, *8*(1), 406.
- Khan, M. M., Sonkar, G. K., Alam, R., Singh, S., Mehrotra, S., & Sonkar, S. K. (2017). Association of ADIPOQ Gene Variant rs266729 with Circulatory Adiponectin Levels in Patients with Type 2 Diabetes in North Indian Population: A Case-Control Study. *Biomedical and Pharmacology Journal*, *10*(1), 407-417.
- Kalehsar, N. S., & Golmohammadi, T. (2015). Association between serum adiponectin and HDL-C in type II diabetic patients. *Global Journal of Health Science*, *7*(2), 243-246.
- Kamenova, P. (2006). Improvement of insulin sensitivity in patients with type 2 diabetes mellitus after oral administration of alpha-lipoic acid. *Hormones-Athens*, *5*(4), 251-258.
- Tuppad, S., Medala, K., Umesh, M., Gaur, A., Ganji, V., Sakthivadivel, V., & Kumar, P. (2022). Serum Adiponectin and Nitric Oxide Levels in Type II Diabetes and Its Correlation With Lipid Profile. *Cureus*, *14*(4), e24613.
- Kocot, J., Dziemidok, P., Kiełczykowska, M., Hordyjewska, A., Szcześniak, G., & Musik, I. (2017). Adipokine profile in patients with type 2 diabetes depends on degree of obesity. *Medical science monitor: international medical journal of experimental and clinical research*, *23*, 4995-5004.
- Karimi, H., Nezhadali, M., & Hedayati, M. (2018). Association between adiponectin rs17300539 and rs266729 gene polymorphisms with serum adiponectin level in an Iranian diabetic/pre-diabetic population. *Endocrine Regulations*, 52(4), 176-184.