Turkish Journal of Physiotherapy and Rehabilitation**; 32(3)**
ISSN 2651-4451 | **e-ISSN** 2651-446X

ASSESSMENT OF VASPIN RS2236242 SNP AND CORRELATION WITH METABOLIC PROFILE IN IRAQI PATIENTS WITH T2DM

Enas D. Neama[1,4], Thana M. Joda[2], Nihad A. Al. Jeboori [3]
[1,2,3] collage of Medicine, Babylon University, Iraq

ABSTRACT

Background: Type 2 diabetes (T2D), formerly known as adult-onset diabetes, is a form of diabetes that is characterized by high blood sugar, insulin resistance, and relative lack of insulin. The aim of this study is investigation of vaspin rs2236242 SNP and correlation with metabolic profile in Iraqi patients with T2DM.

Subjects and Methods: This study includes 90 individuals, the age was ranged between)32- 56(years These subjects were divided into two groups, the first group includes 45 patients with diabetic and the second group includes 45 healthy individuals. Meatabolic profile, FBG,TG,TC,HDL,LDL were done by spectrophotometric methods while rs2236242 SNP of vaspin was done by PCR-ARMS genotyping analysis.

Results: The result shows statistically significant increase ($p<0.01$) in total cholesterol (TC), TG, LDL-C , HDL and FBG in T2DM compare to control group(p-value<0.05). All subjects are categorized depending on the process of fragmentation of amplicon of vaspin being (TT genotype) for homozygous polymorphism and (TA genotype) for heterozygous polymorphism. the comparison between A and T allele frequency (case-control) and evaluated a recessive model of the A allele (TT+TA vs. AA) and a dominant model of T allele(TT vs. TA+AA). Conclusion:

Keywords: Type 2 diabetes Mellitus, Vaspin, metabolic profile, SNP

I. INTRODUCTION

DM is a chronic metabolic disease which effect on Metabolism of carbohydrate, lipid, and protein (1). It is expressed precisely by persistent hyperglycemia (elevated of blood glucose) , resulting from Insufficient secretion of insulin or defects in the action of insulin or summation of both impaired secretion and wrong action of insulin(a hormone product by pancreas) (2) . It is becoming one of the main chronic non- contagious diseases threatening the health of human around the world (3). DM leads to complications in most organs of the human body such as heart, eye, kidney, and nervous system which has resulted in high cost and burden, Therefore, diagnosis of disease in early stages is very essential (4). Type 2 Diabetes Mellitus (T2DM) is complicated, miscellaneous, not autoimmune and multiple gene inheritances metabolic disease condition, in which the body is not able to produce sufficient insulin and characterized by irregular glucose homoeostasis. Its pathogenesis seems to include complicated mutual actions between genetic predisposition and environmental factors. T2DM takes place when impaired insulin resistance (IR) is accompanied by the failure to produce ample amount of β-cell insulin (5). In adults, T2DM is far more common accounting approximately 90% to 95% of all diagnosed cases of diabetes(6), encompasses persons who have IR and usually have relative insulin deficiency at least initially, and frequently throughout their lifetime. These patients do not require insulin treatment to stay a live (7). Vaspin (visceral adipose tissue-derived serine protease inhibitor) was isolated for the first time from visceral adipose tissue in Otsuka Long-Evans Tokushima Fatty rats, which comprises an animal model of abdominal obesity, accompanied by type 2 diabetes mellitus (T2DM) (8). The precise mechanism of vaspin in the body is not well known(9). This may be due to inhibition of proteases that degrade antihyperglycemic and anorexigenic molecules(10) or anti-inflammatory effect (11) Thus far, it is not distinct whether elevated vaspin levels represent a cause or the consequence of T2DM. vaspin was identified as an adipokine with insulin-sensitizing effects, which is predominantly secreted from visceral adipose tissue in a rat model of type 2 diabetes (12). In humans, vaspin expression in terms of mRNA was detected in human visceral and subcutaneous adipose tissue (13), Vaspin is a novel adipocytokine that is supposed to have insulin sensitizing effects (14). The aim of this work to assessment of vaspin rs2236242 SNP and correlation with metabolic profile in Iraqi Patients with T2DM.

II. MATERIALS AND METHODS

Study design:

This study was approved on patients be present at Merjan Teaching Hospital in Babylon province in Hilla city. All samples were collected 11 Oct. 2020 till 23Dec2020. The practical side of the study was performed at the laboratory of the biochemistry department in the faculty of the Medicine / University of Babylon as case-control study. This study includes 90 individuals, the age was ranged between)32- 56(years These subjects were divided into two groups, the first group includes 45 patients with diabetic and the second group includes 45 healthy individuals. All individuals are assessed the status of obesity by the body mass index (BMI), [BMI= weight (kg) / height $(m)^2$].,

Determination of VAS levels:

Human Visceral adipose-specific serine protease inhibitor (vaspin) ELISA Kit employs a two-site sandwich ELISA to quantitate vaspin in samples. An antibody specific for Human vaspin has been pre-coated onto a microplate.

PCR-ARMS analysis

Genomic DNA was extracted from peripheral whole blood of all subjects (OS and NOS) who participating in this study by using the genomic DNA mini kit(Invitrogen™ PureLink™ Genomic DNA Mini Kit) that providing an efficient method for purifying of total DNA from whole and frozen blood. Allele specific PCR was performed by used unique primers for analysis of VAS rs2236242 SNP, as shown in table 1.

Table-1: primers and bands of VAS rs2236242 SNP gene in genotyping analysis

SNP	Primer sequence(5'→3')	Amplicon length
VAS rs2236242	F primer 5'-GGA GGC AGA CCA GGC ACT AGA AA-3' R primer 5'-ACC ATC TCT CTG GCT TCA GGC TTC-3'	174, 248, and 378 bp

PCR was carried out in a total volume 25 µl of reaction mixture with Taqman polymerase and carried by the thermocycler (bio rad) and subjected to denaturation at 94 Cº for 5 min, followed by 30 cycles of 94 Cº for 30 sec, 61.5 Cº for 30 sec and the final extension phase at 72 Cº for 5 min. The final PCR product was electrophoresis by agarose gel (1.5%) and photo documentation the products.

III. STATISTICAL ANALYSIS

Hardy Weinberg equilibrium was used to genotypes estimation. To determine the significant differences between the study groups as related with genotype and allele frequencies by using chi-square test in both patients and control groups.

Results

Clinical characteristics of T2DM group is shown in table 2:

Table-2: Characteristics of T2DM group

CLINIC-PATHOLOGICAL VARIABLES	NO.
Total number of patients	45
Age	
-- <50	24
- ≥50	21
Sex	
- Male	22
- Female	23
Obesity status	2
- underweight	12
- normal weight	21
- Overweight	10
- obese	

The metabolic profile in both T2DM and control group is showing in figure1.

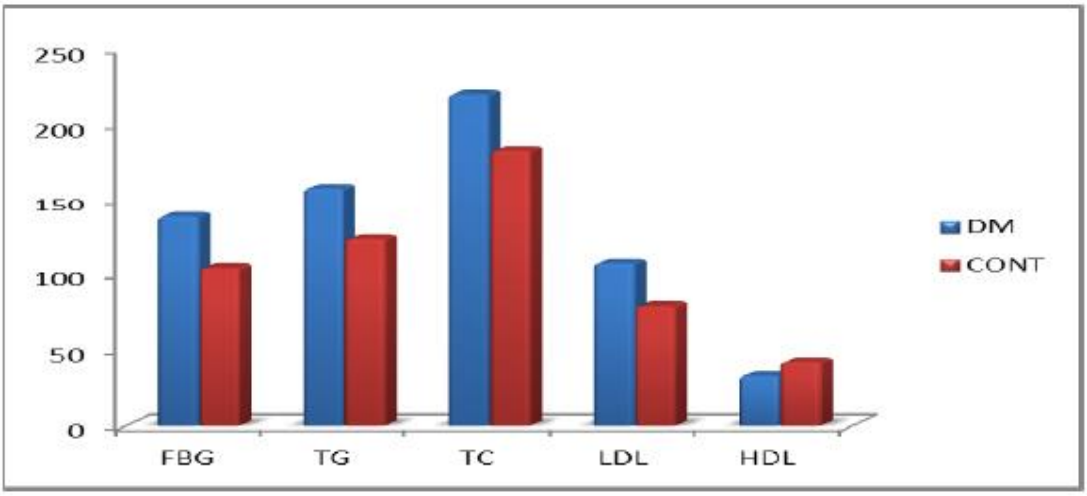

Fig (1): Metabolic profile comparson in patient and control

The results suggesting highly significant differences in VAS levels between T2DM and control group (p-value< 0.05), as showing in table-3:

Table-3: VAS levels in study group

Parameter	Group	Mean ± SD	P-value
VASPIN	Patients group	4.39±0.8	0.001
	Control group	1.12±0.2	

For genotyping analysis, PCR-ARMS used to amplification of target sequence as showing in figure 2.

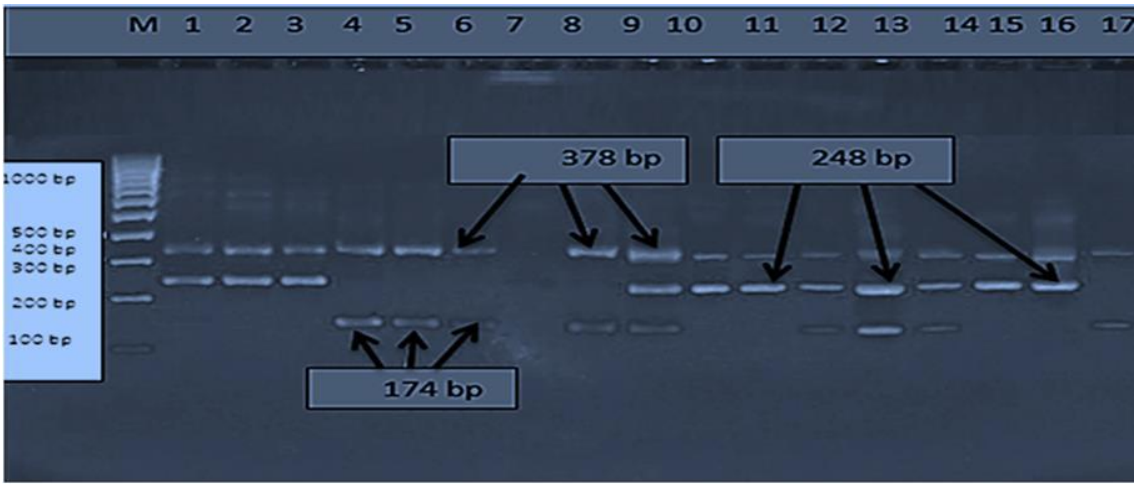

Figure (2): Electrophoresis of amplification fragments for detection of SNP in VAS rs2236242 in both study groups; M: DNA marker (100 bp), lane 4,5,6 and 17 (174 and 378 bp for homozygous TT), lane 1,2,3,10,11,15 and 16 (248 and 378 bp for homozygous AA), lane 9,12,13, and 14(174, 248, and 378 bp for heterozygous TA), and lane 7 NTC.

The results suggesting statistical differences (odd ratio) in CC, CG, and GG between psoriasis and control groups, as showing in table-4:

Table-4: Comparison of VAS rs2236242 genotypes incidence in T2DM and control groups

Genotype	Control n=45 (%)	DM n= 45(%)	Total	OR (CI 95%)	p-value
TT	13 (29)	18 (40)	31	1.573(0.63-3.92)*	0.0117*
TA	25 (55)	22 (49)	47	1.0 (Reference)	
AA	7 (16)	5 (11)	12	1.811(0.69-2.96)*	0.0218*
Total Alleles frequency (p+q=1)	45	45	90		
T	57%	64%		1.94(0.53-3.66)*	0.008
A	43%	36%			

IV. DISCUSSION

VAS is known to play many roles inside the human body. It has a protective role against inflammation, and skin desquamation related to obesity and osteoporosis (15). The serum vaspin concentration remarkably higher in the T2DM group than in the control groups. Our findings confirm that vaspin levels are affected by T2DM. In addition,. Results of the presented study found that levels were significantly higher compared to the control group this study agree with studies of Wei Yang and Yun Li, and et al(16- 18). And dis agree withJian et al., study 2014 (19) found a significant decrease in serum vaspin in type 2 diabetes mellitus patients group than a healthy control group. Other scientists have noted in previous studies that people with type 2 diabetes actually suffer from elevated serum levels of vaspin (20). The present study was carried out to see the association between serum VAS and adipokin that adipokin increase the insulin sensitivity of adipose tissue. The increase of vaspin expression acts like a compensatory mechanism and being a reaction to growing insulin resistance(21). Yet, a study found that high levels of vaspin were a risk factor for the development of T2DM. Dai et al. (2016) found that serum vaspin concentrations were elevated in T2DM patients (22). Genetic study includes 45 diabetic patients and 45 healthy persons. The frequencies for each of the vaspin genotype were found as 40% for TT (n = 18), 49% for TA (n = 22), and 11% for AA(n = 5) in the DM group; 29% for TT (n = 13), 55% for TA (n = 25),and16% for AA (n = 7) in the control group . from figure(4) and table(4) can tell that the genotype TT was high significant in patient than control (p= 0.0117)showed significant homogeneity and the geno type was high significant in control than patient(p= 0.0218). Also, the table shows, the patients and control groups in the Genotypes at all loci were in Hardy- Weinberg equilibrium for genotyping (p+q=1). To evaluate the significance of these results, a Chi-square test was used to investigate the odds ratio (O.R.) and the significance of genotyping and allele frequency. We identified a significant association of rs2236242 A/T with T2D as the TT genotype 49 % high risk and association with increased FBG, BMI and TGlevels. Vaspin rs2236242 TT genotype increases the risk of T2D our study agree with Rathwa and et al (23).

Conflict of interest

No potential conflict of interest relevant to this manuscript was reported.

REFERENCES

1. Iqra Hameed, Shariq R Masoodi, Shahnaz A Mir, Mudasar Nabi, Khalid Ghazanfar, and Bashir. Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition.A Ganai.2015.
2. Chaudhury A, Duvoor C, Dendi R, Sena V, Kraleti S, Chada A, et al. Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. Front Endocrinol (Lausanne). 2017;8:6.
3. Suk WA, Ahanchian H, Asante KA, Carpenter DO, Diaz-Barriga F, Ha E-H, et al. Environmental pollution: an under-recognized threat to childrens health, especially in low-and middle-income countries. Environ Health Perspect. 2016;124(3): 41-45.
4. Latif S, Khan MY, Qayyum A, Qadir J, Usman M, Ali SM, et al. Mobile technologies for managing non-communicable diseases in developing countries. In: Mobile Applications and Solutions for Social Inclusion. IGI Global; 2018. p. 261 87.
5. Moini J. Epidemiology of Diabetes. Elsevier; 2019.

6. 37. Hassan M. Measurement of Prolactin Level among Type2 Diabetic Patients, Aldaraga Health Center, Wad Medani, Gezira State, Sudan (2017). University of Gezira; 2018.
7. Alberti KGMM. The Classifi cation and Diagnosis of Diabetes Mellitus. In: Textbook of Diabetes. 4th editio. A John Wiley & Sons, Ltd., Publication; 2010. p. 24–30.
8. What Is Type 2 Diabetes? Causes, Symptoms, Diagnosis, and Treatments, Written by Lisa M. Leontis RN, ANP-C, Amy Hess-Fischl MS, RD, LDN, BC-ADM, CDE and Jennifer Lutz | Reviewed by Jessica Rodriguez CNP.
9. Suliga E, Wawszczak M, Gluszek S. The roles of vaspin, chemerin, and omentin in the determination of metabolic syndrome. Med Studies. 2018;34(2):160–177
10. Moradi S, Mirzaei K, Abdurahman AA, Keshavarz SA, Hossein Nezhad A (2016) Mediatory effect of circulating vaspin on resting metabolic rate in obese individuals. European Journal of Nutrition 55(3): 1297-1305
11. Bluher M (2012) Vaspin in obesity and diabetes: pathophysiological and clinical significance. Endocrine 41(2): 176-182
12. Wada J (2008) Vaspin: a novel serpin with insulin-sensitizing effects. Expert Opin Investig Drugs 17(3): 327-333
13. Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes Klöting, J Berndt, S Kralisch, P Kovacs… - Biochemical and …, 2006 - Elsevier N.
14. Kloting N, Berndt J, Kralisch S, Kovacs P, Fasshauer M, et al. (2006) Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. Biochem Biophys Res Commun 339: 430–436.
15. Heiker JT. Vaspin (serpinA12) in obesity, insulin resistance, and inflammation. Journal of Peptide Science. 2014;20:299-306...
16. Saalbach A., Tremel J., Herbert D., et al. Anti-inflammatory action of keratinocyte-derived vaspin
17. relevance for the pathogenesis of psoriasis. American Journal of Pathology. 2016; 186(3):639–651.
18. Wei Yang, Yun Li, Tian Tian, Li Wang, "Serum Vaspin Concentration in Elderly Type 2 Diabetes Mellitus Patients with Differing Body Mass Index: A Cross-Sectional Study", BioMed Research International, vol. 2017, Article ID 4875026, 7 pages, 2017.
19. Jian W, Peng W, Xiao S, Li H, Jin J, Qin L et al., Role of serum vaspin in progression of type 2 diabetes: a 2-year cohort study. PLoS One. 2014; 14;9:e 94763.
20. Hida K., Poulsen P., Teshigawara S., et al. Impact of circulating vaspin levels on metabolic variables in elderly twins. Diabetologia. 2012; 55(2):530–532.
21. Hamzah H. Kzar , Moshtak A. wtwt , Moaed E. Al- Gazally). Study the Glucose Transport, Angiogenesis and Apoptosis Behavioral through Chemotherapy Treatment According to Receptors Status in Women with Breast Cancer. Indian Journal of Forensic Medicine & Toxicology;2020, 14(3), 2555-2559.
22. Mena Y. Abd, Hamzah H. Kzar, Manal M. Murad. Assessment of Alpha Ketoglutarate Dependent Dioxygenase Levels in Different Genotypes of ALKBH9 Gene in Patients with T2DM in Babylon Province. Indian Journal of Public Health Research & Development, October 2019, 10(10).2125-30.
23. Dai R., Dong Z., Qian Y., Han Y. Obese type 2 diabetes mellitus patients have higher serum vaspin concentrations. Journal of Diabetes. 2016;8(3):445–447. doi: 10.1111/1753-0407.12365.