

## Genetic Polymorphisms of Carnosine Synthase -1(ATPGD1) and Serum Carnosine Levels in Relation to Cardiovascular Diseases in Iraqi Type 2 Diabetics

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### Abstract

Diabetes mellitus (DM) is a metabolic disorder specified by persistent hyperglycemia that occurs when  $\beta$ -cell insulin production became unable to control blood glucose with deterioration of response for insulin. The consequent development of DM complications with increased risk for many diseases caused by damaged variety of biological systems, including blood vessels, eyes, kidneys, heart, and nerves. Carnosine level in human serum is affected by several enzymes like carnosine synthase-1 (responsible of carnosine synthesis), thus any genetic polymorphisms in these enzymes could have an impact on enzymes levels and therefore serum carnosine level with consequent increased risk for many diseases.

This study was aimed to estimate the occurrence of Carnosine synthase-1 single nucleotide polymorphism (rs 1790733) on chromosom11 (intron –variant) in Iraqi type 2 diabetics with and without cardio vascular diseases and its correlation with serum levels of carnosine & carnosine synthase-1 and their association with CVD as T2DM complication.

To achieve this aim Enzyme Linked Immunosorbent Assay (ELISA) specific kits were used to estimate serum levels of carnosine & carnosine synthase-1. High resolution melt technique (HRM) was applied for detecting gene polymorphism of carnosine synthase-1 gene (ATPGD1).

The results showed that gene polymorphism of SNP (rs1790733) increased the chance of CVD in T2DM patients by 84.5% by its effect to decrease the mean of serum levels of carnosine by decreased carnosine synthase-1 level that may correlate with the development of CVD in T2DM patients.

**Key words:** Carnosine ,Carnosine synthase -1, gene polymorphism,T2DM.

تعدد الأشكال الجينية للكارنوسين سينثيز-1 ومستويات الكارنوسين في المصل وعلاقته بأمراض القلب والأوعية الدموية في مرضى السكري من النوع الثاني العراقيين  
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### الخلاصة

داء السكري هو اضطراب استقلابي محدد بفرط سكر الدم المستمر الذي يحدث عندما يصبح إنتاج الأنسولين في خلية بيتا غير قادر على التحكم في جلوكوز الدم مع تدهور الاستجابة للأنسولين. التطور اللاحق لمضاعفات مرض السكري مع زيادة خطر الإصابة بالعديد من الأمراض التي تسببها مجموعة متنوعة من الأجهزة البيولوجية التالفة، بما في ذلك الأوعية الدموية والعينين والكلية والقلب والأعصاب. يتأثر مستوى الكارنوسين في مصل الدم بالعديد من الإنزيمات مثل كارنوسين سينثيز-1 (المسؤول عن تخليق الكارنوسين)، وبالتالي فإن أي تعدد الأشكال الجينية في هذه الإنزيمات يمكن أن يكون له تأثير على مستويات الإنزيمات وبالتالي مستوى كارنوسين المصل مع ما يترتب على ذلك من مخاطر متزايدة للعديد من الأمراض. هدفت هذه الدراسة إلى تقدير حدوث كارنوسين سينثيز-1 (rs1790733) SNP على الكروموسوم 11 (إنترن-متغير) في مرضى السكري من النوع الثاني العراقيين مع وبدون أمراض القلب والأوعية الدموية وارتباطه بمستويات مصل كارنوسين و كارنوسين سينثيز-1 وارتباطهم بأمراض القلب والأوعية الدموية كمضاعفات لداء السكري من النوع الثاني. ولتحقيق هذا الهدف، تم استخدام طريقة المقايمة المتميز المرتبط بالإنزيم لتقدير مستويات مصل الكارنوسين والكارنوسين سينثيز-1. تم تطبيق تقنية الذوبان عالية الدقة للكشف عن تعدد الأشكال الجيني لكارنوسين سينثيز. أظهرت النتائج أن تعدد الأشكال الجيني للطفرة (rs1790733) زاد من فرصة الإصابة بأمراض القلب والأوعية الدموية في مرضى داء السكري من النوع الثاني بنسبة 84,5% بتأثيره على خفض متوسط مستويات الكارنوسين في الدم عن طريق انخفاض مستوى الكارنوسين سينثيز-1 الذي قد يرتبط بتطور الأمراض القلبية الوعائية في مرضى داء السكري من النوع الثاني.

الكلمات المفتاحية: كارنوسين، كارنوسين سينثيز-1، تعدد الأشكال الجيني، داء السكر من النوع الثاني.

## Introduction

Diabetes mellitus (DM) is a metabolic disorder specified characterize by persistent hyperglycemia as a result of reduction of  $\beta$ -cell insulin production and/ or insulin resistance usually with deterioration of response to insulin. The diabetic complications occurs due to increased risk for several diseases induced as a result of damaged to a variety of biological systems, including blood vessels, eyes, kidneys, heart, and nerves <sup>(1,2,3)</sup>. Type 2 diabetes mellitus (T2DM) is significantly the more frequent type of DM, doubles or quadruples the risk of death from cardiovascular disease or stroke and, it is associated with both micro- and macrovascular complications, due to accelerated atherosclerosis predisposing to severe peripheral vascular diseases<sup>(4,5)</sup>. Many factors are known to promote the development of endothelial dysfunction underlying cardiovascular diseases such as : hypercholesterolemia, diabetes mellitus, metabolic syndrome, hypertension, aging ( accumulation of advanced glycation end products), oxidative stress, proinflammatory cytokines <sup>(6)</sup>.

Carnosine is a natural dipeptide ( $\beta$ -alanyl-L-histidine) expressed in both the central nervous system as well as the periphery in both vertebrate and invertebrate organisms. Also it's found in several tissues most notably in muscle as appreciable fraction of the total water-soluble nitrogen-containing compounds<sup>(7)</sup>. Carnosine is synthesized starting from its component amino acids  $\beta$ -alanine and L-histidine via the activity of the ATP-dependent enzyme carnosine synthetase-1, the origins of the two amino acids forming carnosine are very different. The non-proteinogenic amino acid  $\beta$ -alanine is synthesized by the liver, primarily via uracil and thymine degradation, while L-histidine, a proteinogenic essential amino acid, is not synthesized de novo in human beings and must thus be ingested by food<sup>(8)</sup>. Carnosine is synthesized by a cytosolic amino acid ligase, carnosine synthase-1 (CARNS1; also known as ATP-grasp domain-containing protein 1, ATPGD1; EC 6.3.2.11), in the presence of GABA (i.e. in the central nervous system), <sup>(9,10)</sup>. Several biological and physiological functions have been attributed to carnosine, including: anti-inflammatory and antioxidant, antiglycating activities, and as a modulator of mitochondrial metabolism. Hence, this dipeptide can be used to prevent and treat diseases such as diabetes, neurodegenerative diseases, diseases of the sense organs and cancers. It may also cure or alleviate many other disorders because of its wide spectrum of activities <sup>(11)</sup>.

Carnosine may also improve exercise performance and vasodilator response, thereby protecting from ischemic tissue injury, it may have

a role as an adjunct treatment for peripheral vascular disease alongside typical exercise and surgical interventions, thus can be used in high risk individuals to prevent atherogenesis<sup>(12)</sup>. Besides carnosine functional properties that are specific to muscle and excitable tissues to act as a quencher for Advanced Glycation Endproducts and ALE (Advanced Lipid peroxidation Endproducts (AGE/ALE) precursors Reactive Carbonyl Species (RCS), which are highly reactive aldehydes derived from oxidative and non-oxidative modifications of sugars and lipids. Consistently, carnosine was found to be effective in several disease models in which glyco/lipoxidation plays a central pathogenic role<sup>(13)</sup>. Other studies have shown an anti-diabetic effect of carnosine supplementation and an endogenous antioxidant is documented to accelerate healing of wounds and ulcers by increased expression of growth factors and cytokines genes involved in wound healing. In vitro studies with human dermal fibroblasts and microvascular-endothelial cells had shown that carnosine increases cell viability in presence of high glucose, such effects and others are depending on its role as an antioxidant and a precursor for histamine synthesis, which provide evidence for a possible therapeutic use of carnosine in diabetic wound healing<sup>(14,15)</sup>. Therefore, evidences indicated that carnosine has roles in treatment and protection against many diseases like Alzheimer, Parkinsonism, neuropathy, nephropathy and also it can alleviate aging-related vascular diseases, such as atherosclerosis and related complications in type 2 diabetes, in other words carnosine may prevent development of cardiovascular disease linked to vascular calcification, including atherosclerosis, diabetes, and chronic renal failure <sup>(16,17)</sup>. Some genetic polymorphisms specially single nucleotide polymorphisms (SNPs) may lead to diseases like Rheumatoid Arthritis (RA), nephrotic syndrome, decreased response to treatment <sup>(18,19)</sup>. Whereas, genetic polymorphisms related to enzymes that affects serum levels of related proteins like carnosine synthase-1 and carnosine level could lead to increased risk of many diseases as well as the related complications like in case of T2DM<sup>(20)</sup>. In this research the Single Nucleotide Polymorphisms(SNPs), which are related to one of the enzymes that are related to carnosine metabolism, specifically carnosine synthase-1 (ATPGD1); was studied to investigate their possible relation to development of CVD in type2 diabetics.

## Subjects and Methods

This is a case-controlled study was conducted as a multicenter study in Baghdad/Iraq, Including The national center for Diabetic Research and Ibn Al-Bitar Center for Cardiac Surgery, during the period from April /2022 till October /2022.

An overall 150 Iraqi adult subjects of both genders were enrolled in the study, with age range of (35 - 65 years), were divided into three groups. Type 2 diabetic patients were already on oral antidiabetic treatment. The diabetic patients <sup>(100)</sup> were diagnosed by specialized physician (at least 5 years ago), based

on the American Diabetes association (ADA) criteria <sup>(21)</sup>, the **First group** :included T2DM patients without complications, **second group** :included T2DM patients with cardiovascular complications(angina, or myocardial infarction, or stroke),with ECG based diagnosis, supported by information from their medical records and family history of cardiovascular diseases (CVD)<sup>(22)</sup>.

In addition to fifty apparently healthy subjects in the **third group** to serve as controls (Table -1). Subjects having chronic kidney or liver disease were not included, nor those with autoimmune disease nor vegetarian subjects.

**Table 1. Descriptive data for participants in the study**

Parameters	(Group-1) T2DM without complications	(Group-2) T2DM with CVD	(Group-3) Healthy control
Number	50	50	50
Gender (F/M)	25/25	25/25	25/25
Age(years)	44.00 ± 6.52 <sup>a</sup>	44.04 ± 6.59 <sup>a</sup>	45.84 ± 7.89 <sup>a</sup>
BMI(Kg/M <sup>2</sup> )	27 ± 4.25 <sup>a</sup>	26.36 ± 3.20 <sup>a</sup>	27.26 ± 1.7 <sup>a</sup>
FSG(mg/dl)	233.34 ± 65.33 <sup>a</sup>	230.56 ± 59.67 <sup>a</sup>	97.74 ± 9.99 <sup>b</sup>
HbA <sub>1c</sub> (%)	9.59 ± 2.15 <sup>b</sup>	10.72 ± 2.25 <sup>a</sup>	5.32 ± 0.50 <sup>c</sup>
TG (mg/dl)	141.50 ± 29.31 <sup>b</sup>	371.58 ± 96.39 <sup>a</sup>	119.8 ± 44.19 <sup>b</sup>
VLDL(mg/dl)	30.04 ± 17.35 <sup>b</sup>	65.70 ± 19.22 <sup>a</sup>	23.66 ± 12.89 <sup>b</sup>
LDL(mg/dl)	102.51 ± 25.09 <sup>b</sup>	128.94 ± 19.21 <sup>a</sup>	92.52 ± 29.08 <sup>c</sup>

Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different, FSG=Fasting serum glucose, HbA<sub>1c</sub>=Glycated Hemoglobin, TG=Triglycerides, VLDL=Very low density lipoprotein, LDL=Low density Lipoprotein.

### Specimen collection and handling

About three milliliters of fasting venous blood specimens were obtained from each participant after about 12 hours of fasting. One ml of the collected blood was transferred to an ethylene diamine tetraacetic acid (EDTA) tube and stored at (+2 to +8°C) for analysis of HbA<sub>1c</sub> and, 1 milliliter of blood was collected into another EDTA tube for DNA extraction and genotyping by High Resolution Melt (HRM) real time PCR. The remainder of blood sample was transferred to a gel tube to collect serum after clotting and centrifugation at (3000 rpm) for 5 minutes. Serum was stored as aliquots at -20°C for analysis of carnosine, carnosine synthase-1 by enzyme-linked immunosorbent assay (ELISA)<sup>(23,24)</sup>.

### Polymorphism study steps

#### Genomic DNA extraction

Genomic DNA was extracted from the peripheral blood leukocytes (frozen EDTA blood samples) by Easy Pure Blood Genomic DNA Kit

and then measurement the concentration and purity of DNA by a Nano drop UV spectrophotometer by which the optical density of DNA (1.5 µl) was measured at two wavelengths (260 and 280 nm)<sup>(25)</sup>. In most samples, DNA preparation gave A260/A280 ratio between 1.8 and 2.0, which is considered to be suitable for further analysis in detection gene polymorphisms. The measurement of DNA concentration for most of the samples was in the range of (25-118) ng/ml.

#### The primers preparation

The primers applied in this study are listed in Tables 2 below, were lyophilized, and they were liquefied in the nuclease-free water to give a final concentration of 100 pmol/µl as stock solution. The stock was kept at -20°C, to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free deionized distilled water to reach a final volume of 100 µl, the primers were supplied by Alpha DNA company/ USA.

**Table 2. The primers of carnosine synthase-1 gene for HRM technique**

SNP	Primer Sequence	Tm (°C)
rs1790733(ATPGDA-1)	F- 5' CTGTGCTGCTGGAGGGTG 3' R- 5' GGCTGCCCTTTCTGTAGAA 3'	56

HRM =High-Resolution Melting analysis.

#### High-resolution melting technique

The genetic variations were investigated for SNPs detection for (rs1790733) using HRM real-time PCR.

#### Assay principle

As a logical progression from the real-time monitoring of PCR reactions high-resolution melting (HRM) analysis is a homogeneous, incredibly robust method for genotyping, sequence, and mutation scanning in DNA samples <sup>(26)</sup>. The HRM method was enabled not only to provide its apparatus or software but also to provide third-generation dyes that made this method more accurate, such as the fluorescent Eva green dye, which can bind to dsDNA. One of the advantages of this dye is that it is non-toxic to cells, has no trans-membrane permeability, and is non-mutagenic. It can be used in large quantities to obtain the largest percentage of saturation of dsDNA and thus more brilliance and greater accuracy in the measured signals <sup>(27)</sup>. High-Resolution Melting analysis (HRM) for genetic analysis (Polymorphism analysis) was performed using a Rotor gene Q Real-time CYTO PCR System(QIAGEN), scaling temperature for amplification of DNA from 55 to 95 °C then HRM analysis was done with 0.1°C within a wavelength (470-510 nm). The 2xTransStart Tip Green qPCR

SuperMix was used for SNPs sequences determination. qPCR-HRM was utilized on triple synthetic controls to determine allelic differences, and differential curves (DC) and, normalized melting curves (NMC) were created using the HRM Tool included in the integrated software (Rotor gene 4.4).

#### Reaction Components of HRM

The reaction components of HRM analysis was shown in Table 3 below.

**Table 3. The Reaction Components of HRM Analysis for Genotype Using Quantitative Real-Time PCR**

Reaction Components	Volume (µl)
2x TransStart@Tip Green qPCR	10
Super Mix 10	5
Nuclease free water	1
Forward primer	1
Reverse primer	1
DNA	3
Final volume	20µl

HRM =High-Resolution Melting analysis.

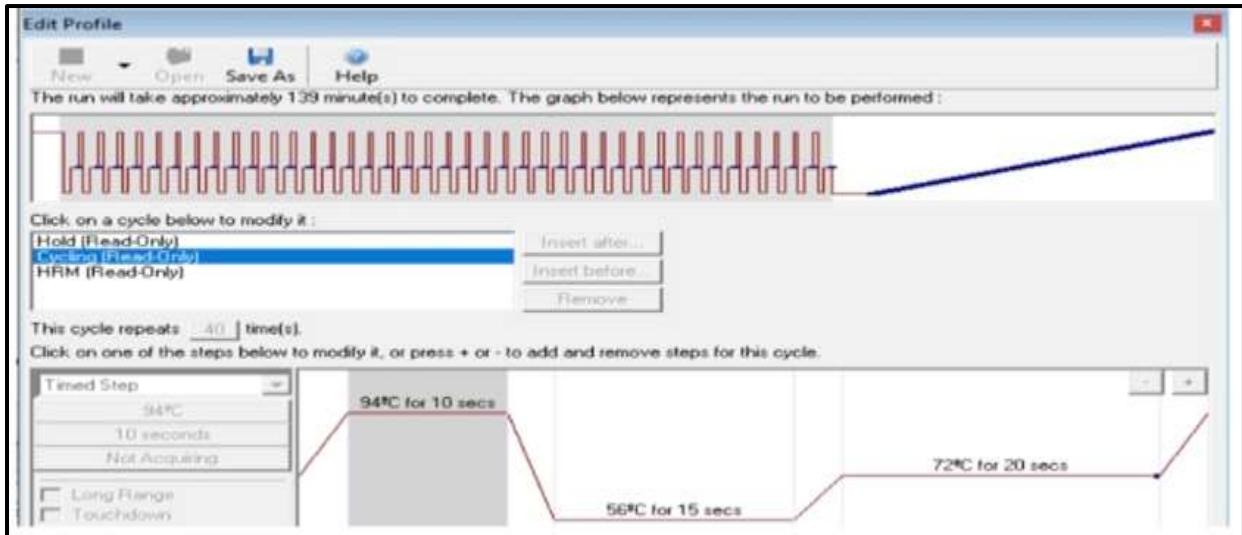
#### Thermal profile of HRM technique

The details of HRM technique are illustrated in Table 4 and Figure1 below:

**Table 4. Thermal Profile of the HRM Technique**

Step	Temperature(°C)	Time(Second)	NO. Cycle
Enzyme Activation	94	30	1
Denaturation	94	5	40
Annealing	56	15	40
Extension	72	20	40
HRM	65-95	0.1 degree for 2 sec	1

HRM =High-Resolution Melting analysis



**Figure 1. The thermal profile for hrn analysis in a real-time PCR device .**

The annealing degree was 56°C and using EVA green dye, the reaction took about 120 minutes.

**Statistical Analysis**

**Included the followings:**

T-test and, the least significant difference (LSD) test (Analysis of Variation ANOVA) were used to compare significantly between means, Means followed by different letters are significantly different according to Duncan’s multiple range comparisons (DMRTs), and Means followed by the same letter are not significantly different. A Chi-square test was used to significantly compare percentages.

Pearson correlation coefficient (r) was used to calculate the correlation between parameters. Values of probability in this study were considered significance as below:

\*: (P ≤0.05) means Significant., \*\* (P≤0.01) means Highly Significant. NS: means non-significant. Using Version 11.63 of the WINPEPI computer program to evaluate the statistical significance of the P values calculated with Fisher's exact test; as well as the Odds Ratio that was assessed by a special  $\chi^2$  formula (Abramson, 2

**Results and Discussion**

The results of current study demonstrated highly significant differences for both: carnosine, carnosine synthase-1, measured in serum for the patients and control groups as illustrated in Table 5 & Figures 2, 3 b.

**Table 5. Serum Carnosine and Carnosine Synthase-1 Levels**

Groups	Carnosine (ng/ml)	Carnosine synthase-1 (ng/ml)
(Group-1)T2DM with CVD	0.19 ± 0.13 <sup>b</sup>	0.54 ± 0.18 <sup>b</sup>
(Group-2)T2DM without complications	0.23 ± 0.10 <sup>b</sup>	0.43 ± 0.23 <sup>b</sup>
(Group-3) Healthy control	1.14 ± 0.43 <sup>a</sup>	1.85 ± 0.46 <sup>a</sup>

Means followed by different letters are significantly different according to Duncan’s multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different

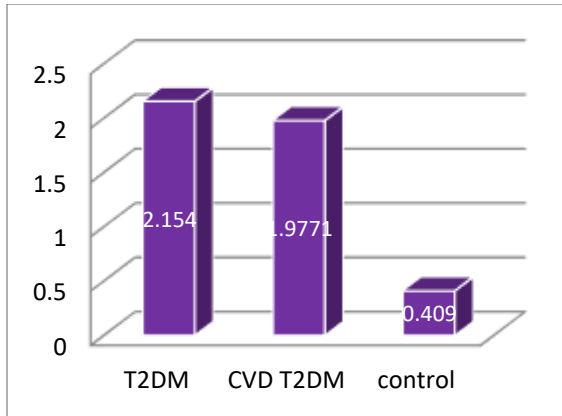


Figure 2. Serum Carnosine Levels in studied groups

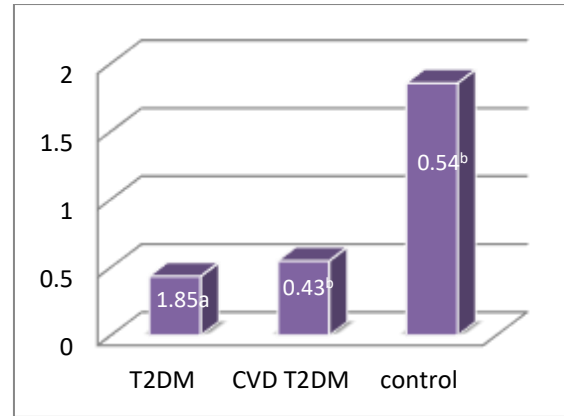


Figure 3. Serum Carnosine synthase-1 Levels in studied groups.

The markers measured in this study have different sensitivity and specificity percentage that show the efficiency of the studied markers and the dependence on them as an indicator of disease as shown in Table-6 and Figures (4, 5) below

Table 6. Receiver Operating Characteristic curve data of the studied markers

Parameters	AUC	Explanation	P-value	The best Cut off	Sensitivity %	Specificity%
Carnosine	0.98	Excellent	0.001	0.4785	98	97
Carnosine synthase-1	0.98	Excellent	0.001	0.9615	96	99

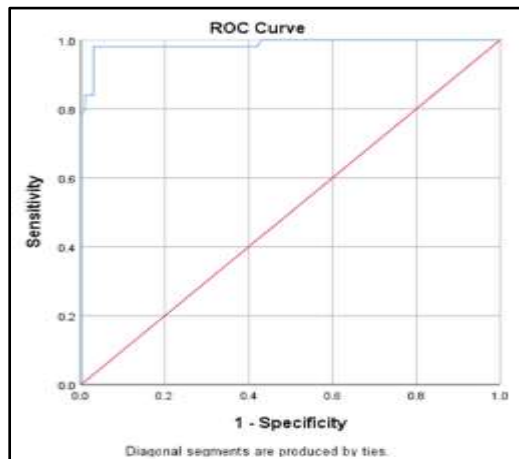


Figure 4. Receiver Operating Characteristic curve of the carnosine biomarker.

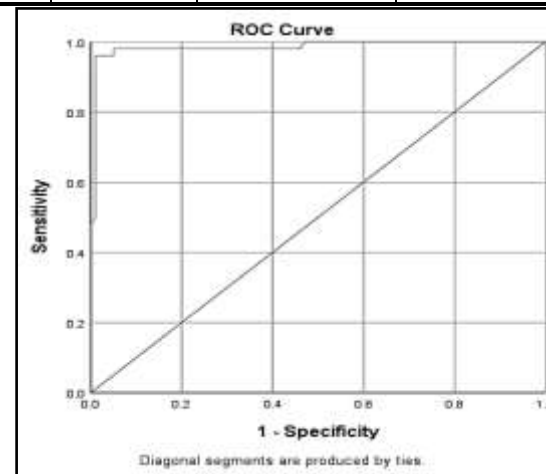


Figure 5. Receiver Operating Characteristic curve of the carnosine synthase-1 biomarker

The results clarified a strong correlation between serum level of carnosine and carnosine synthase-1, as shown in Table 7 below:

Table 7. Correlation of studied parameters

Parameters		Carnosine	Carnosine synthase-1
Carnosine	Pearson Correlation	1	0.765**
	Sig. (2-tailed)		0.000
Carnosine synthase-1	Pearson Correlation	0.765**	1
	Sig. (2-tailed)	0.000	

Genotyping results show that there were non-significant differences in genotype distribution and allele frequency between T2DM patients and the control groups considering carnosine synthase-1 SNP (rs1790733), as Fisher's exact probability was 0.2 as showed in Table 8, furthermore the outcome of (T2DM with CVD) group genotyping showed that (AA) genotype (wild type) non-significant difference (p-value = 0.5), and (AG) hetero genotype showed highly significant difference (p -

value 0.03) that increase risk of disease in carrying subjects to (60.4%). Also (GG) mutant genotype showed highly significant difference (p - value 0.001) between subjects, odds ratio (6.47) and increase the risk of CVD to (84.5%), and allele distribution results appear the important role of G allele in increase CVD complication odds ratio (2.02) in patients (T2DM with CVD) group in this study as clarified as in Table 9.

**Table 8. Genotypes and Allele Frequency of carnosine synthase -1 SNP (rs1790733) in T2DM& Control**

Genotypes Group rs1790733	Study Group		Odds Ratio	CI 95%	Fisher's exact probability	Etiological fraction	Prevented Fraction
	Patients (N=50)	Control (N=50)					
AA	19 (38.00%)	20 (40.00%)	0.92	0.41 to 2.07	0.84		8.1%
AG	22 (44.00%)	26 (52.00%)	0.73	0.33 to 1.61	0.4		27.5%
GG	9 (18.00%)	4 (8.00%)	2.52	0.72 to 9.98	0.2	60.4%	
<b>Alleles Distribution</b>							
A	60(60.00%)	66(66.00%)	0.77	0.43 to 1.38	0.4		22.7%
G	40(40.00%)	34(34.00%)	1.29	0.70 to 2.40	0.4	22.7%	

**Table 9. Genotypes and Allele Frequency of carnosine synthase -1 SNP ( rs 1790733) in CVD-T2DM and Control**

Genotypes Group rs1790733	Study Group		Odds Ratio	CI 95%	Fisher's exact probability	Etiological fraction	Prevented Fraction
	Patients (N=50)	Control (N=50)					
AA	17 (34.00%)	20 (40.00%)	0.77	0.34 to 1.76	0.5		22.7%
AG	15 (30.00%)	26 (52.00%)	0.4	0.17 to 0.91	0.03		60.4%
GG	18 (36.00%)	4 (8.00%)	6.47	2.06 to 23.71	0.001	84.5%	
<b>Alleles Distribution</b>							
A	49(49.00%)	66(66.00%)	0.49	0.28 to 0.88	0.01		50.5%
G	51(51.00%)	34(34.00%)	2.02	1.14 to 3.59	0.01	50.5%	

The results of current study showed no observational effect of SNP ( rs 1790733) on carnosine serum levels that indicate non-significant differences (p-value > 0.05) between three genotype within each groups as presented in Table 10 below, on the other

hand the Table 11 showed highly significant difference (p = 0.01) in serum level of carnosine synthase -1 in variant genotype in control group when compare the three groups of study.

**Table 10. Serum carnosine levels (ng/ml) with variant genotype of carnosine synthase -1 SNP**

Groups	rs1790733	Mean± SD	No.	p-value
T2DM	AA	0.22±0.06	19	0.7
	AG	0.24±0.13	22	
	GG	0.25±0.06	9	
CVD -T2DM	AA	0.15±0.08	17	0.2
	AG	0.20±0.14	15	
	GG	0.23±0.14	18	
Control	AA	1.21±0.50	20	0.4
	AG	1.12±0.36	26	
	GG	0.93±0.51	4	

Data are presented as Mean ± Std. Deviation.

**Table 11. Serum carnosine synthase-1 levels (ng/ml) with variant genotype of carnosine synthase -1 SNP**

Groups	rs1790733	Mean± SD	NO.	p-value
T2DM	AA	0.38±0.08	19	0.3
	AG	0.48±0.33	22	
	GG	0.40±0.06	9	
CVD- T2DM	AA	0.61±0.17	17	0.1
	AG	0.52±0.16	15	
	GG	0.50±0.20	18	
Control	AA	1.91 ± 0.37	20	0.01 **
	AG	1.91±0.40	26	
	GG	1.19±0.82	4	

\*\*= highly significant difference (p <0.01). Data are presented as Mean ± SD.

Type 2 Diabetes increases the risk of many diseases like nephropathy, retinopathy, and cardiovascular disease<sup>(28)</sup>, the previous studies showed that carnosine has several important biological activities, through its impact on age-related diseases such as cardiovascular disease, DM, cancer and neurological problems and play an important role in improving the functional capability in ischemia circumstances<sup>(29,30)</sup>. Carnosine prevents CVD by different mechanisms through its anti-inflammatory, anti-glycating, and antioxidant properties<sup>(31)</sup>.

The results of the current study clarified the correlation between carnosine synthase -1 (rs 1790733) gene polymorphism on chromosome 11 (intron-variant) with serum level of carnosine and carnosine synthase enzyme-1 and their effects on CVD, increased the chance of disease occurrence by 84.5% in subjects carrying (GG mutant genotype) in T2DM with CVD group. The increase in oxidative stress and inflammatory factors in hyperglycemia (Hauck AK. et al 2019)<sup>(32)</sup>, that could be the leading cause in the deterioration of T2DM and consequent complications, besides other risk factors to CVD associated with diabetes which arise as metabolic disorders and insulin resistance, such as: increase in serum level of fasting glucose, HbA1c, TG and

VLDL, LDL) (Table-1). Several previous studies ensure the role of carnosine in normalizing plasma glucose level and reduced insulin levels after an oral glucose intake & proven that carnosine lowered fasting glucose level, serum levels of TG, enhanced lipid metabolism & improved glycemic control (decreased HbA1c, insulin resistance, increased insulin secretion)<sup>(32,33)</sup> indirectly by activation signaling cascade of nuclear factor erythroid 2-related factor 2 (Nrf2), which enhances endogenous antioxidant and anti-carboxylation defensive mechanisms<sup>(34)</sup>.

The results clarified that the SNP had no effect or play a role in the occurrence of CVD as a complication of T2DM. Furthermore, the studied SNP had been associated with increased the chance of disease occurrence by 84.5% in subjects carrying (GG mutant genotype) in T2DM with CVD group, while showed a decrease in carnosine, carnosine synthase-1 (ATBGD1) serum levels in both of the diabetic groups, caused by indirect effect of the SNP on hyperglycemia in the diabetic groups that clarify the decrease in carnosine levels in the same groups as well.

A study by Zhao, et al. have proven the effect of carnosine synthase-1 in protection from myocardial ischemia and other heart disease<sup>(35)</sup>, polymorphisms



in other genes that encode enzymes related to carnosine, like carnosinase enzyme isoform1&2, can also affect carnosine levels in serum of T2DM leading to increase the occurrence of diabetic complications (Zhang S, et al. 2020)<sup>(36)</sup>. Gene polymorphism of SNP (rs1790733) was chosen in this study to investigate its role in development CV complications in T2DM according to our knowledge studied before. However the current study still represent only the first steps toward a better understanding of the genetic factors that influence the development of CVD as one of the serious complications affecting T2DM patients, so further studies in this regard are necessary before implementation of research findings into practice.

### Conclusion

Gene polymorphism of SNP (rs1790733) increased the chance for developing CVD in T2DM patients by decreasing serum level of carnosine synthase-1 leading to decrease serum level of carnosine causing a deterioration of DM complication specially CVD.

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### Conflicts of Interest

The authors declare no conflicts of interest.

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### Ethics Statements

Ethical approval with the number (RECAUBCP9112021A) on 29 Nov, 2021, was obtained from the Scientific and Ethical Committee in the College of Pharmacy / University of Baghdad.

### Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Shatha H Ali; data collection: Haneen Subhee Shaheed; analysis and interpretation of results: Shatha H Ali, Haneen Subhee Shaheed; draft manuscript preparation: Shatha H Ali, Haneen Subhee Shaheed. All authors reviewed the results and approved the final version of the manuscript.

### References

1. Raheem MF, Ali SH, Shareef LG. Impact of serum levels of vitamin D on lipid profiles, glycemic indices, and insulin resistance in obese type 2 diabetes patients: An observational study. *F1000Research*. 2022 Sep 6;11(1002):1002.
2. Salih BH, Ali SH, Allehibi KI. Serum Aldosterone Levels in Patients With Diabetic Nephropathy in Relation to Vascular Calcification. *Iraqi Journal of Pharmaceutical*

3. Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2019 Jun 11;28(1):53-63.
3. Khamis AS, Ali SH, Al-Lehibi KI. The correlation between serum total adiponectin and hemoglobin in type 2 diabetic patients without microalbuminuria. Age (years). *Iraqi Journal of Pharmaceutical Sciences*(P-ISSN 1683-3597 E-ISSN 2521-3512).2012;55(2.231):53-23.
4. Abdulrahman Z, Alatrakji MQ, Al-Maliky AA, Hussein KI, Hussain SA. Influence of Metformin Dose and Treatment Adherence on Glycemic Control, Adiposity, and Cardiovascular Risk Markers in Iraqi Patients with T2DM. *Journal of the Faculty of Medicine Baghdad*. 2022;64(4):218-26.
5. Yonus SS, AlKataan MA. Mitochondrial Copies Number and Some Renal Function Biomarkers in Type 2 Diabetes Mellitus on Metformin. *Iraqi Journal of Pharmaceutical Sciences* (P-ISSN 1683-3597 E-ISSN 2521-3512). 2022 Dec 23;31(2):33-8.
6. Incalza MA, D'Oria R, Natalicchio A, Perrini S, Laviola L, Giorgino F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascular pharmacology*. 2018 Jan 1;100:1-9.
7. Banerjee, S., Mukherjee, B., Poddar, M. K., & Dunbar, G. L. Carnosine improves aging-induced cognitive impairment and brain regional neurodegeneration in relation to the neuropathological alterations in the secondary structure of amyloid beta (A $\beta$ ). *Journal of Neurochemistry*.2021; 158(3), 710-723.
8. Menini S, Iacobini C, Fantauzzi CB, Pugliese G. L-carnosine and its derivatives as new therapeutic agents for the prevention and treatment of vascular complications of diabetes. *Current medicinal chemistry*. 2020 Apr 1;27(11):1744-63.
9. Kwiatkowski S, Kiersztan A, Drozak J. Biosynthesis of carnosine and related dipeptides in vertebrates. *Current Protein and Peptide Science*. 2018 Aug 1;19(8):771-89.
10. Albrecht T, Schilperoort M, Zhang S, Braun JD, Qiu J, Rodriguez A, Pastene DO, Krämer BK, Köppel H, Baelde H, de Heer E, Anna Altomare A, Regazzoni L, Denisi A, Aldini G, van den Born J, Yard BA, Hauske SJ Carnosine attenuates the development of both type 2 diabetes and diabetic nephropathy in BTBR ob/ob mice. *Sci Rep*. 2017; 7:44492.
11. Zhao J, Shi L, Zhang LR. Neuroprotective effect of carnosine against salsolinol induced Parkinson's disease. *Experimental and therapeutic medicine*. 2017 Jul 1;14(1):664-70.
12. Diniz FC, Hipkiss AR, Ferreira GC. The Potential Use of Carnosine in Diabetes and

- Other Afflictions Reported in Long COVID Patients. *Front Neurosci.* 2022;16:898735. Published 2022 Jun 22.
13. Huang Y, Wang J, Luo M, Yan D, Zhang C. Carnosine attenuates vascular smooth muscle cells calcification through mTOR signaling pathway. *Aging Medicine.* 2020 Sep;3(3):153-8.
  14. Baye E, Ukropec J, De Courten MP, Vallova S, Krumpolec P, Kurdiova T, Aldini G, Ukropcova B, De Courten B. Effect of carnosine supplementation on the plasma lipidome in overweight and obese adults: a pilot randomised controlled trial. *Scientific Reports.* 2017 Dec 12;7(1):1-7.
  15. Khudhur SS, Saleh ES, Alosami MH. The Impact of rs767455 and rs1061622 Polymorphisms on Treatment Outcomes in Iraqi Ankylosing Spondylitis Patients Taking Etanercept. *The Egyptian Journal of Hospital Medicine.* 2023 Jan 1;90(2):3488-94.
  16. Schmöhl, F., Peters, V., Schmitt, C. P., Poschet, G., Büttner, M., Li, X. & Kroll, J. CNDP1 knockout in zebrafish alters the amino acid metabolism, restrains weight gain, but does not protect from diabetic complications. *Cellular and Molecular Life Sciences,* 2019:76(22), 4551-4568.
  17. Caruso G, Fresta CG, Grasso M, Santangelo R, Lazzarino G, Lunte SM, Caraci F. Inflammation as the common biological link between depression and cardiovascular diseases: Can carnosine exert a protective role?. *Current Medicinal Chemistry.* 2020 Apr 1;27(11):1782-800.
  18. Jameel MG, Alhakeem ZM, Al-Osami MH. Prevalence of AGER gene polymorphism in post menopause Iraqi sample with Osteoporosis and osteopenia in type 2DM. *Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512).* 2022 Dec 24;31(2):202-10.
  19. Ali R, Baban R, Ali S. The association between PON1 gene polymorphisms (Q192R and L55M) and nephrotic syndrome in Iraqi children. *Baghdad Journal of Biochemistry and Applied Biological Sciences.* 2021 Jul 21;2(03):120-32.
  20. Caruso G, Caraci F, Jolivet RB. Pivotal role of carnosine in the modulation of brain cells activity: Multimodal mechanism of action and therapeutic potential in neurodegenerative disorders. *Progress in neurobiology.* 2019 Apr 1;175:35-53.
  21. Sureshkumar K, Durairaj M, Srinivasan K, Goh KW, Undela K, Mahalingam VT, Ardianto C, Ming LC, Ganesan RM. Effect of L-Carnosine in Patients with Age-Related Diseases: A Systematic Review and Meta-Analysis. *Frontiers in Bioscience-Landmark.* 2023 Jan 18;28(1):18.
  22. Wang C, Yuan Y, Zheng M, Pan AN, Wang M, Zhao M, Li Y, Yao S, Chen S, Wu S, Xue H. Association of age of onset of hypertension with cardiovascular diseases and mortality. *Journal of the American College of Cardiology.* 2020 Jun 16;75(23):2921-30.
  23. Kohl TO, Ascoli CA. Immunometric double-antibody sandwich enzyme-linked immunosorbent assay. *Cold Spring Harb Protoc.* 2017;1(6):458–62.
  24. Alan H. B. Wu. *Immunochemical Techniques.* In: Michael L. Bishop, Edward P. Fody LES, editor. *Clinical Chemistry Principles, Techniques, and Correlations.* 8th ed. Philadelphia:Wolters Kluwer; 2018. p. 437–80.
  25. *Technical Manual ReliaPrep™ Blood gDNA Miniprep System Instructions for Use of Products A5080, A5081 and A5082.*
  26. Wittwer CT, Reed GH, Gundry CN, Vandersteen JG, Pryor RJ. High-resolution genotyping by amplicon melting analysis using LCGreen. *Clinical chemistry.* 2003 Jun 1;49(6):853-60.
  27. Ameen IA, Saleh E, Mhaibes SH, Taha K, Dawood DA, Kamil HS. Evaluation of some inflammatory cytokines and Glycated hemoglobin in uncontrolled type 2 diabetes mellitus with nephropathy. *Indian Journal of Forensic Medicine & Toxicology.* 2020 Apr 29;14(2):1628-32.
  28. AL-Qadhi HI, AL-Kinani TA, Obeed AA. Effect of Dapagliflozin on hemoglobin level in heart failure patients with chronic kidney disease and/or diabetes. *Journal of the Faculty of Medicine.* 2022;4(64), Pages 203-207
  29. Caruso G, Fresta CG, Grasso M, Santangelo R, Lazzarino G, Lunte SM, Caraci F. Inflammation as the common biological link between depression and cardiovascular diseases: Can carnosine exert a protective role?. *Current Medicinal Chemistry.* 2020 Apr 1;27(11):1782-800.
  30. Huang Y, Wang J, Luo M, Yan D, Zhang C. Carnosine attenuates vascular smooth muscle cells calcification through mTOR signaling pathway. *Aging Medicine.* 2020 Sep;3(3):153-8.
  31. Hauck AK, Huang Y, Hertzell AV, Bernlohr DA. Adipose oxidative stress and protein carbonylation. *Journal of Biological Chemistry.* 2019 Jan 25;294(4):1083-8.
  32. Calabrese V, Scuto M, Salinaro AT, Dionisio G, Modafferi S, Ontario ML, Greco V, Sciuto S, Schmitt CP, Calabrese EJ, Peters V. Hydrogen sulfide and carnosine: modulation of

- oxidative stress and inflammation in kidney and brain axis. *Antioxidants*. 2020 Dec 18;9(12):1303.
33. de Courten B, Kurdiova T, de Courten MP, Belan V, Everaert I, Vician M, Teede H, Gasperikova D, Aldini G, Derave W, Ukropec J. Muscle carnosine is associated with cardiometabolic risk factors in humans. *PloS one*. 2015 Oct 6;10(10):e0138707.
34. Matthews JJ, Dolan E, Swinton PA, Santos L, Artioli GG, Turner MD, Elliott-Sale KJ, Sale C. Effect of carnosine or  $\beta$ -alanine supplementation on markers of glycemic control and insulin resistance in humans and animals: a systematic review and meta-analysis. *Advances in nutrition*. 2021 Nov;12(6):2216-31.
35. Zhao J, Conklin DJ, Guo Y, Zhang X, Obal D, Guo L, Jagatheesan G, Katragadda K, He L, Yin X, Prophan MA. Cardiospecific overexpression of ATPGD1 (carnosine synthase) increases histidine dipeptide levels and prevents myocardial ischemia reperfusion injury. *Journal of the American Heart Association*. 2020 Jun 16;9(12):e015222.
36. Zhang S, Xu J, Cui D, Jiang S, Xu X, Zhang Y, Zhu D, Xia L, Yard B, Wu Y, Zhang Q. Genotype distribution of CNDP1 polymorphisms in the healthy Chinese Han population: association with HbA1c and fasting blood glucose. *Journal of Diabetes Research*. 2020 Jul 18;2020.