"Association of CTRP and chemokine ligand-2 in Egyptian diabetic women with or without CAD"

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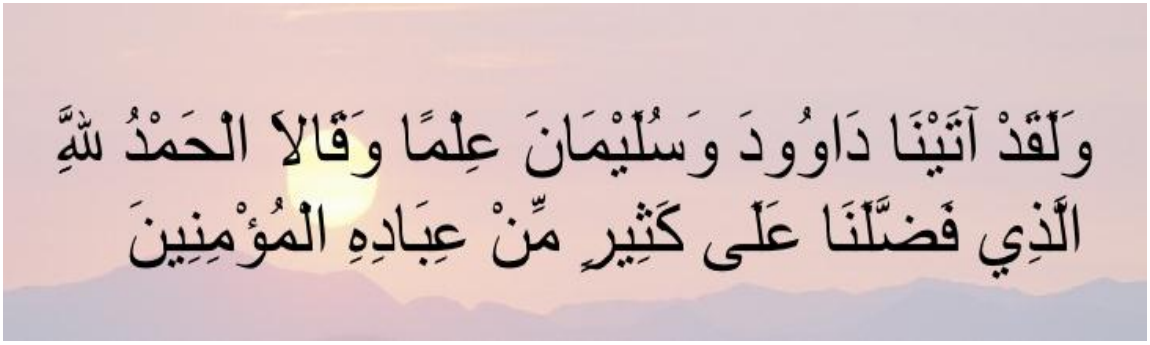
2019

بسم الله الرحمن الرحيم



صدق الله العظيم

سورة النمل آية ١٥

ACKNOWLEDGMENTS

Acknowledgments

First and foremost, I would like to thank "**God**", without his help and grace I would have never been able to accomplish anything in work and life and for granting me the opportunity to expand my knowledge through this research.

I owe my deepest sincere gratitude and respect for accomplishing this work to my supervisor **Prof. Dr. Hala Osman El-Mesallamy, Professor and Head of Biochemistry Department, Faculty of Pharmacy, Ain Shams University,** for her continuous support, guidance, patience, encouragement and enlightening advice throughout the whole work. All the knowledge I have in research is because of her and I hope one day I can transfer that knowledge to others as well.

I would like to thank **Ass. Prof. Dr. Marwa Shabayek, Biochemistry Department, Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt,** my supervisor for all her efforts, co-operation, scientific guidance and support, she gave to put this work.

I would like to thank **Prof. Dr. Mohamed Hesham El Hefnawy, Dean of the National Institute of Diabetes and Endocrinology** for his keen guidance, following up the patients and facilitating samples collection.

I would like to thank **Dr. Mostafa Sayed Abdelghany, Department of cardiology, Hussein Medical hospital** for his tremendous help by following up the patients and facilitating samples collection.

I am gravely thankful to the Biochemistry Department in both pharmacy faculties of Ain Shams University and Future University for their help to complete this research.

I would like to thank all our study volunteers, participated physicians, the nursing staff, and laboratory technicians for their assistance in performing the mentioned study.

No words can express my gratitude towards my family, especially my **Father and Mother**, without their love, care, guidance, prayers and support, after **God**, I would have never been able to achieve anything throughout my life. I would like to thank my **Brothers** for being there for me. May God bless all of them.

Last but not least, I would like to thank my best friend **Lecturer Mae Seleem** and my friends **Samar Hussein,** and **Suzan Magdy** for all their support throughout my research.

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PUBLICATION RELATED TO THE THESIS

PLOS ONE

RESEARCH ARTICLE

Role of CTRP3, CTRP9 and MCP-1 for the evaluation of T2DM associated coronary artery disease in Egyptian postmenopausal females

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OPEN ACCESS

Citation: Ahmed SF, Shabayek MI, Abdel Ghany ME, El-Hefnawy MH, El-Mesallamy HO (2018) Role of CTRP3, CTRP9 and MCP-1 for the evaluation of T2DM associated coronary artery disease in Egyptian postmenopausal females. PLoS ONE 13 (12): e0208038. https://doi.org/10.1371/journal.pone.0208038

Editor: Jonathan M Peterson, East Tennessee State University, UNITED STATES

Received: July 18, 2018

Accepted: November 9, 2018

Published: December 17, 2018

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Data Availability Statement: The data used in this study have been deposited in Harvard Dataverse and may be accessed at doi.org/10.7910/DVN/I1DIKC.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abstract

C1q complement/tumor necrosis factor (TNF)—related protein (CTRP) family comprises of 15 proteins that posses important implications in energy homeostasis, infection and inflammation. However, their roles in diabetes mellitus (DM) and its vascular complications have not been completely assessed. This works aims to study the association of two CTRPs; 3 and 9, with pro-inflammatory cytokine monocyte chemoattractant protein-1 (MCP-1), and biochemical parameters of type 2 diabetes (T2D), dyslipidemia and coronary artery disease (CAD). **Methods**: Biochemical markers and serum levels of CTRPs and MCP-1 were measured in 86 postmenopausal females. Subjects were divided over four groups; 13 apparent healthy subjects as control (group I), 29 patients with CAD (group II), 29 patients with T2D ≥5 years (group III) and 15 patients with CAD secondary to T2D (group IV). Serum CTRP3, CTRP9, MCP-1 and insulin were measured by ELISA. **Results**: Serum CTRP3 levels were found to be significantly higher in group III and IV, whereas, it was significantly lower in group II on comparing to group I. While, CTRP9 levels were significantly decreased in group II, III and IV on comparing to group I. MCP-1 levels were found to be significantly increased in groups II, III and IV on comparison with group I. Both CTRPs were significantly negatively correlated with each other. While MCP-1 was significantly correlated negatively to CTRP9. **Conclusion**: This study associates the possible role of CTRP3, CTRP9 and MCP-1/CCL2 in the diagnosis/prognosis of CAD complication in T2D postmenopausal females.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease, its development involves several pathogenic processes [1]. Collectively all of these processes results in loss of β-cell mass and/or

function that are clinically manifested as hyperglycemia [2]. The number of diabetic patients in 2015 were estimated to be around 415 million people and expected to reach 642 million by the year 2040, which represents an exponential increase mainly in developing countries[[3].

Among the several types of DM, type 2 diabetes (T2D) is a progressive pandemic that accounts for more than 90% of all cases, representing an indisputable threat to the public health[[4]. T2D is a complex polygenic disorder which is a result of the combination of hereditary components and secondary contributing factors which include; obesity, lifestyle, birth weight and stress [5].

Obesity represents one of the prominent risk factors for T2D and its complications, it shows an equivalent increase in its global prevalence [6]. Visceral obesity and adipose tissue causes, metabolic disturbance, leading to various obesity associated co-morbidities including; metabolic syndrome, T2D, hyperlipidemia and heart diseases [7]. In addition, obesity is correlated with T2D through proinflammatory cytokines (adipokines), insulin sensitivity, abnormal fatty acid metabolism, and intracellular disturbances; mitochondrial dysfunction and endoplasmic reticulum stress [8,9].

In 2004, the CTRP super-family emerged as novel anti-inflammatory adipokines with important metabolic roles [10]. The CTRP family is composed of 16 members including adiponectin (APN), like APN, the CTRP family members were believed to play a vital role in energy homeostasis, through altering insulin sensitivity specifically in the muscles and liver, all of which regulates whole-body energy metabolism thus, CTRPs could be novel pharmacological targets in T2D, obesity and inflammatory diseases [11].

A member of the CTRP family and one of the closest functional homolog of APN with a potent anti-inflammatory, antidiabetic and cardioprotective adipokine is CTRP3 (also known as cartonectin, cartducin, CORS-26) [9,10]. Another member is CTRP9, which plays a role in lowering blood glucose levels, with a potent vasorelaxation effect as its vasoactive potency is about three folds higher than that of APN [12]. However, CTRP9 has not been fully investigated in human subjects suffering from DM.

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the C-C chemokine family, a potent chemotactic factor for monocytes with potent proinflammatory action attributing to many inflammation mediated diseases, such as T2D and coronary artery disease (CAD) [13]. Interestingly, the proinflammatory action of MCP-1/CCL2 was found to be blocked by CTRP3 [11]. However the relationship between the anti-flammatory adipokine CTRP9 and proinflammatory chemokine MCP-1/CCL2 has not been studied along with its role in T2D and CAD.

The current study was designed to develop effective diagnostic and prognostic strategies through evaluating the changes in the levels of CTRP3, CTRP9 and MCP-1/CCL2 in females suffering from either T2D only, CAD only, and CAD secondary to T2D. In addition, correlation between CTRP3, CTRP9, MCP-1/CCL2 with each other and with other routine biochemical parameters were assessed to explain how these possible markers correlate with the diagnosis/prognosis of the patients suffering from T2D alone, CAD alone and the development of CAD in T2D patients.

Material and methods

Subjects

This study was approved by the Medical ethics committee of Ain shams University, a signed informed consent was obtained from all subjects prior to participation in the study. The study complies with all the regulations and recommendations of the declaration of Helsinki.

Table 1. Clinical and laboratory characteristics of the studied groups.

Groups/Parameters	Group(I) (n = 13)	Group(II) (n = 29)	Group(III) (n = 29)	Group(IV) (n = 15)
Age (Years)	51.15±1.82	56.10±0.80	50.48±1.38[b]	56.67±1.63[c]
BMI (kg/m^2)	26.33±0.25	29.89±0.10[a]	29.50±0.05[a]	30.46±0.19[a,b,c]
FBG (mg/dl)	97.46±2.37	96.45±1.54	197.79±3.22[a,b]	189.33±4.26[a,b]
Hb_{A1c} %	5.15±0.08	5.176±0.07	8.476±0.15[a,b]	8.73±0.21[a,b]
Insulin (pmol/l)	7.31±0.62	9.17±1.83[a]	13.69±1.28[a,b]	14.80±0.76[a,b]
Duration of T2D (years)	———	———	7.09±0.29	8.40±0.46[c]
HOMA-IR	1.69±0.13	4.52±0.41[a]	6.69±0.62[a,b]	7.00±0.48[a,b]
QUICKI	0.35±0.01	0.31±0.01[a]	0.30±0.004[a]	0.29±0.002[a,b]
TC (mg/dl)	173.38±3.19	237.55±4.52[a]	257.69±4.76[a,b]	271.67±8.07[a,c]
TG (mg/dl)	143.62±1.82	179.2±1.66[a]	178.66±1.89[a]	191.60±4.68[a,b,c]
LDL-C (mg/dl)	99.77±1.48	182.83±2.09[a]	181.14±1.99[a]	191.73±2.75[a,b,c]
HDL-C (mg/dl)	52.33±1.78	39.31±0.70[a]	38.41±0.77[a]	28.93±1.07[a,b,c]
LDL-C/HDL-C ratio	1.92±0.08	4.62±0.10[a]	4.76±0.11[a]	6.73±0.33[a,b,c]
TC/HDL-C ratio	3.38±0.14	6.10±0.15[a]	6.69±0.13[a]	9.53±0.44[a,b,c]
CTRP3 (ng/ml)	130.38±7.12	80.31±3.05[a]	211.34±5.75[a,b]	186.93±4.02[a,b,c]
CTRP9 (ng/ml)	304.46±9.60	194.90±3.34[a]	126.76±2.95[a,b]	101.40±5.70[a,b,c]
MCP-1(pg/ml)	190.54±6.12	469.45±6.12[a]	331.86±11.41[a,b]	676.27±28.37[a,b,c]

Values are expressed in terms of (Mean ± SEM).

Group I: Apparent healthy control; Group II: patients with CAD; Group III: Patients with type 2 diabetes; Group IV: patients with CAD secondary to type 2 diabetes. BMI: body mass index; FBG: fasting blood glucose; HbA1c: glycated hemoglobin; TG: triglycerides; TC: total cholesterol; LDL-c: low density lipoprotein cholesterol; HDL-c: high density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment—Insulin resistance; QUICKI—quantitative insulin sensitivity check index

[a]: Significantly different from control group I at $p<0.05$

[b]: Significantly different from group II at $p<0.05$

[c]: Significantly different from group III at $p<0.05$

https://doi.org/10.1371/journal.pone.0208038.t001

About 86 subjects were divided into four groups, as follows: Group I included (13) apparent healthy subjects as control with average body mass index (BMI) (**26.33±0.25**). Group II included (29) patients with CAD with average BMI (**29.89±0.10**). Group III included (29) patients with T2D $\geq$ 5 years with average BMI (**29.50±0.05**), group IV included (15) patients with CAD secondary to T2D with average BMI (**30.46±0.19**). The range of BMI between: 18.50–24.99, was classified as normal. While BMI 25–29.99 kg/m^2 was classified as overweight (non obese) subjects, and BMI $\geq$ 30 kg/m^2 was classified as obese subjects[14]. Blood samples were withdrawn from patients in the National Institute of Diabetes and Endocrinology (NIDE), (Cairo, Egypt) and the intensive care unit (ICU) of the cardiology department of Al-Hussein Teaching Hospital (Cairo, Egypt). All the subjects are postmenopausal females. Characteristics of subjects are represented in Table 1.

Medical history was collected from all subjects including; duration of DM (at least 5 years), familial history of DM, type of CAD, medication list, history of any disease and surgical procedures. All subjects were physically examined. CAD was previously diagnosed using cardiac angiogram. All diabetic patients were treated using 500mg metformin twice daily.

The following exclusion criteria were used for all study participants: All patients were free of chronic liver diseases, type 1 DM (T1D), acute or chronic renal disease, hyperthyroidism, disorders in pituitary gland, malignancy, autoimmune disease, and inflammatory diseases. Patients were not taking any anti-inflammatory drugs, as well as other medications that may affect the heart.

Methods

Blood sampling. Whole blood samples were collected after an overnight fast from all 86 subjects. Blood collected was split into three portions; the first portion was used to measure fasting plasma glucose (FPG) and collected in Na fluoride containing vacutainer tubes. The second portion was collected on EDTA containing vacutainer tubes in order to measure glycated hemoglobin (HbA_1c %). The third portion of blood was centrifuged to separate serum for the measurement of: insulin, lipids [triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C)] and the serum levels of CTRP3, CTRP9 and MCP-1. All routine work analyses were measured in the same day of the blood collection, the remaining samples were stored at -80˚C till time of assay for insulin, CTRP3, CTRP9 and MCP-1.

Laboratory assessment. Patient's demographics and medical history were obtained from the patients file or during the patients visit. BMI was calculated using the standard formula (weight (kg)/height (m^2)). Insulin level was measure using BioTina GmbH, Germany ELISA kit (Cat No: E 2035). Levels of FBG (Cat. No: GL 13 20), $Hb_{A_1}c$% (Cat. No: k031296) and levels of TG (Cat. No: TR 20 30), TC (Cat. No: CH 12 20), HDL-C (Cat. No: CH 12 30),and LDL-C (Cat. No: CH 12 31) were measured using Biodiagnostic Co., Egypt kits. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: [fasting blood glucose (mg/dL)] × [fasting blood insulin (μU/mL)/405]. For calculating Quantitative Insulin Sensitivity Check Index we used the following equation (QUICKI: 1/(ln (insulin)+ln (glucose)). The serum levels of CTRP3, CTRP9 and MCP-1 were measured using Aviscera biosciences, USA ELISA kits (Cat. No: SK00082-07 for CTRP3, SK00081-02 for CTRP9 and SK00220-01 for MCp-1).

Statistical analysis

All results were expressed as mean ± standard error of mean (M ± S.E.M). Analysis of variance (ANOVA) was used to compare different groups and to compare individual groups post Hoc LSD was applied. Kolmogorov smirnov test was conducted to ensure the normal distribution of the data and our data met the null hypothesis of the normal distribution. The correlation between the parameters was done using Pearson correlation test which is an extended parametric analysis Simple and multiple linear stepwise regression analyses were done to study the association between each of CTRP3, CTRP9 and MCP-1 with other biochemical parameters. In the multiple linear stepwise regression analysis, the independent variables included demographic factors and other biochemical variables (BMI, age, duration of diabetes, FBG, $Hb_{A_1}c$ %, insulin, TC, TG, HDL-C, LDL-C, TC/ HDL-C risk ratio, HDL-C /LDL-C risk ratio, HOMA-IR and QUICKI), all of which were associated with the examined dependent variable (CTRP3, CTRP9 and MCP-1/CCL2) in univariate analysis. Windows based SPSS statistical package (SPSS version 20.0, SPSS Inc, Chicago, IL) was used to perform all statistical analyses. *P*-values < 0.05 were considered significant.

Results

The power analysis done to power our study, used CTRP3 level as the primary outcome. The effect size d = (2.96975) was calculated based upon the results of Choi et al (2012) for the difference between normal and T2D subjects[15]. The effect size (d) was converted to effect size (f = 1.4849) using effect size converter designed by Jamie DeCoster (2012) to allow for calculating sample size for comparison between the four groups because there was no relevant studies to the present study. Using alpha (α) level of (5%) and Beta (β) level of (20%) i.e. power = 80%;

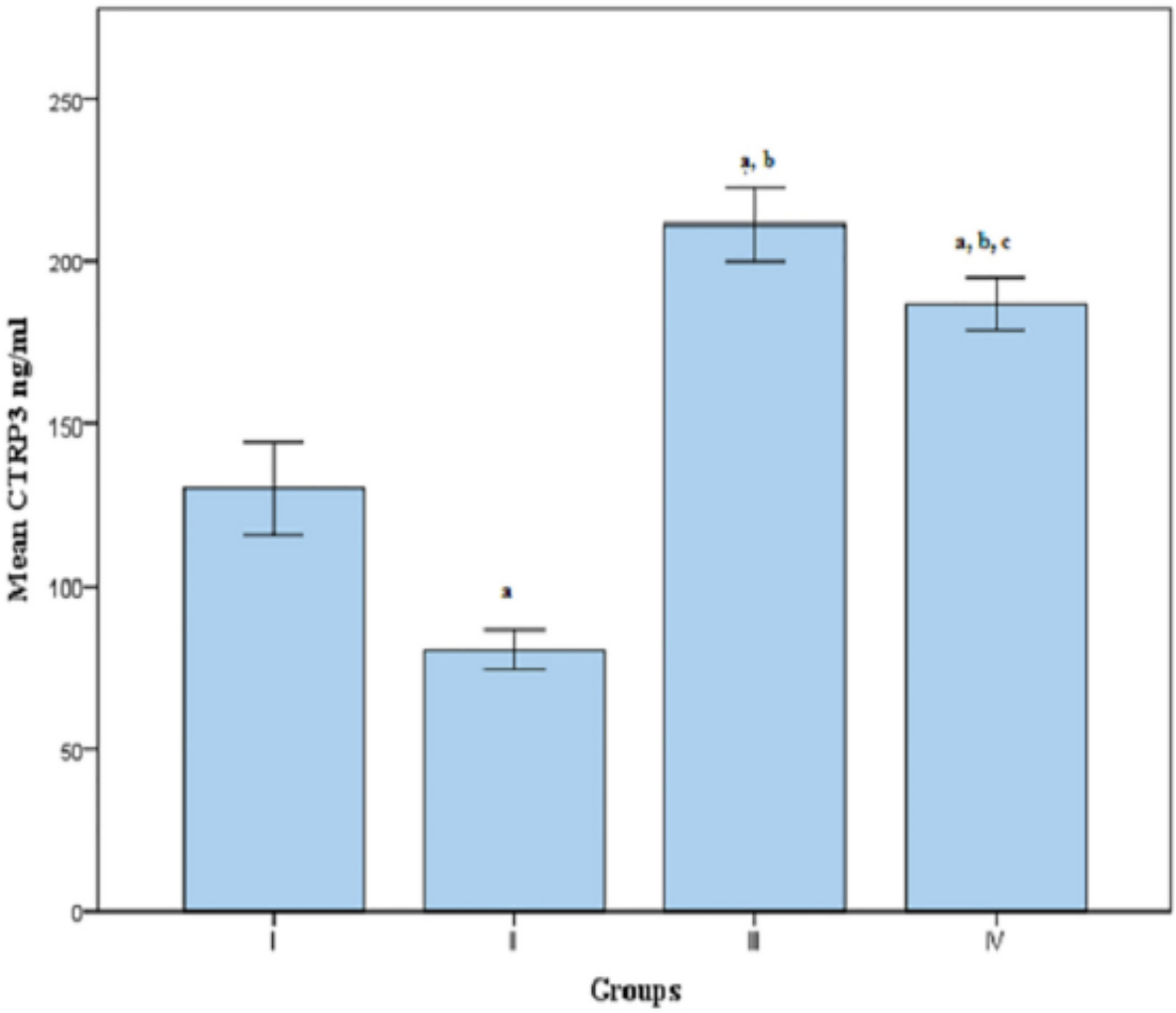

Fig 1. Mean serum CTRP 3 levels in groups I, II, III and IV. Group(I):Apparent healthy control, Group(II):Patients with CAD, Group(III):Patients with T2DM, Group(IV):Patients with CAD secondary to T2DM, a: Significantly different from group I at p<0.05, b: Significantly different from group II at p<0.05, c: Significantly different from group III at p< 0.05, CTRP3: C1q complement/tumor necrosis factor (TNF)—related protein 3.

https://doi.org/10.1371/journal.pone.0208038.g001

the minimum estimated sample size was 12 subjects per group. Sample size calculation was performed using G* Power Version 3.1.9.2.

The clinical characteristics and serum levels of CTRP3, 9 and MCP-1of the studied groups are shown in Table 1. BMI, FBG, $Hb_{A1}c$ %, insulin, HOMA-IR, QUICKI and all lipids profile parameters showed higher levels in groups II, III and IV as compared to control group except HDL-C and QUICKI.

Serum CTRP3 level was found to be significantly higher in group III and group IV whereas, it was significantly lower in group II when compared to group I. In addition, its levels were significantly decreased in group IV when compared to group III. Moreover, CTRP3 level was further elevated significantly in group III when compared to group II at $p<0.05$[Table 1, Fig 1].

Regarding serum CTRP9 level, it was found to be significantly decreased in group II, III and IV when compared to group I. Meanwhile, its level was significantly decreased in group

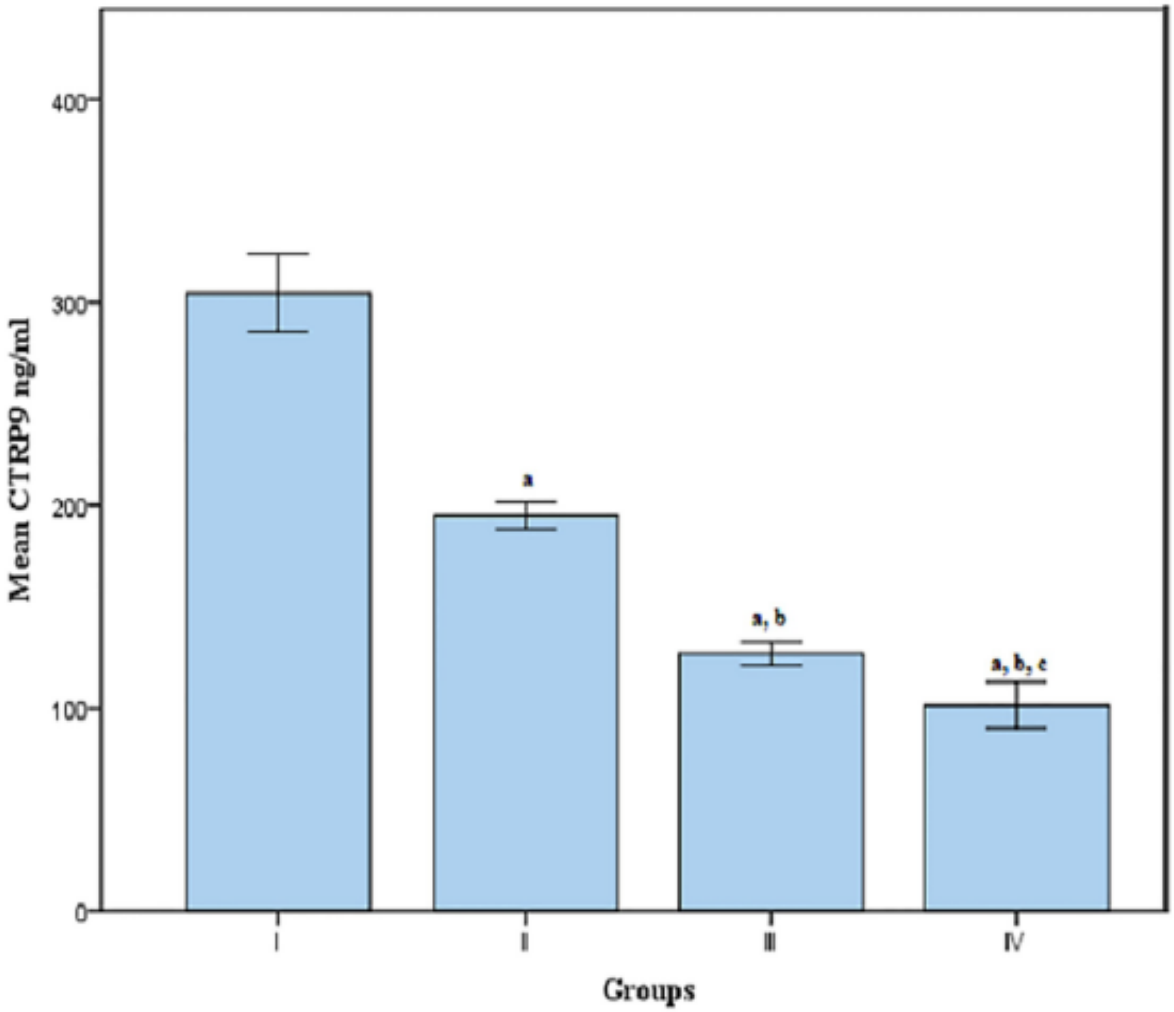

Fig 2. Mean serum CTRP9 levels in groups I, II, III and IV. Group(I):Apparent healthy control, Group(II):Patients with CAD, Group (III):Patients with T2DM, Group(IV):Patients with CAD secondary to T2DM, a: Significantly different from group I at $p<0.05$, b: Significantly different from group II at $p<0.05$, c: Significantly different from group III at $p<0.05$, CTRP9: C1q complement/tumor necrosis factor (TNF)—related protein 9.

https://doi.org/10.1371/journal.pone.0208038.g002

IV, when compared to group II and III. Moreover, CTRP9 level was significantly decreased in group III when compared to group II at $p<0.05$[Table 1, Fig 2].

Regarding serum MCP-1 level, it was found to be significantly increased in group II, III and IV when compared to group I. Meanwhile, its level was significantly increased in group IV, when compared to group II and III. Moreover, MCP-1 level was significantly decreased in group III when compared to group II and IV at $p<0.05$[Table 1, Fig 3].

On performing simple linear regression using CTRP3 as the dependent variable and other biochemical parameters as the independent variables, CTRP3 was found to be significantly positively correlated with other independent variables including; FBG, $Hb_{A1}c$ %, T2D duration, TC, TC/HDL-C, and HOMA-IR while, significantly negatively correlated with age, HDL-C, QUICKI and CTRP9 at $p<0.05$ [Table 2, Fig 4]. While, on performing multiple linear stepwise regression analysis using CTRP3 as dependent variable with other independent

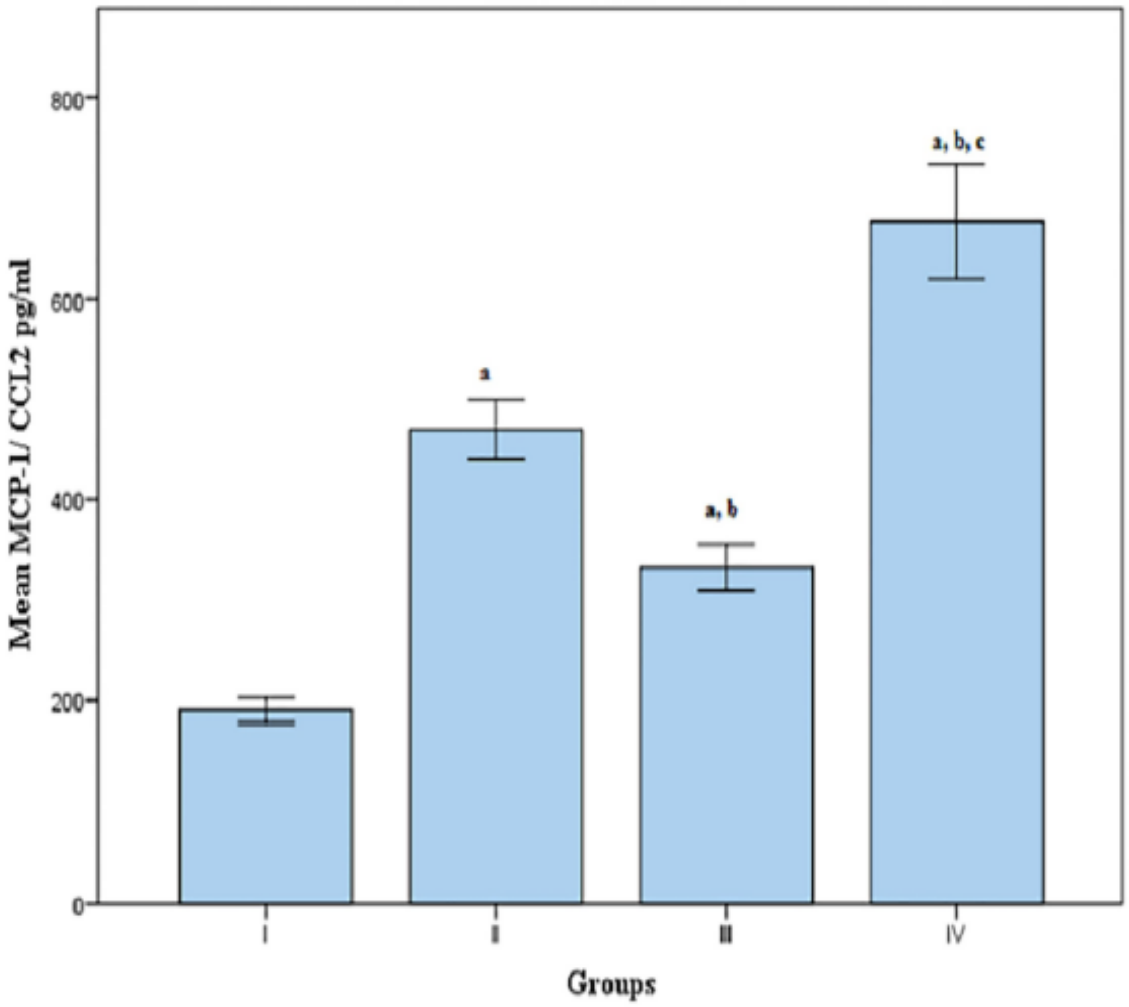

Fig 3. Mean serum MCP-1/ CCL2 3 levels in groups I, II, III and IV. Group(I):Apparent healthy control, Group(II):Patients with CAD, Group(III):Patients with T2DM, Group(IV):Patients with CAD secondary to T2DM, a: Significantly different from group I at $p<0.05$, b: Significantly different from group II at $p<0.05$, c: Significantly different from group III at $p<0.05$, MCP-1: Monocyte chemoattractant protein-1.

https://doi.org/10.1371/journal.pone.0208038.g003

variables only FBG (β = 0.276, P = 0.007), $Hb_{A1}c$ % (β = 0.413, P = 0.002) and T2D duration (β = 0.384, P = 0.003), remained significantly associated with CTRP3 [Table 3].

On performing simple linear regression using CTRP9 as the dependent variable and other biochemical parameters as the independent variables, CTRP9 was found to be significantly positively correlated with HDL-C and QUICKI while, significantly negatively correlated with other independent variables including; BMI, age, duration of diabetes, FBG, $Hb_{A1}c$ %, TC, TG, LDL-C, TC/ HDL-C, HDL-C/LDL-C, HOMA-IR, CTRP3 and MCP-1/CCL2 [Table 2, Figs 4 and 5]. Moreover, on performing multiple linear stepwise regression analysis using CTRP9 as dependent variable with other independent variables only LDL-C (β = -0.215, P = 0.012), FBG (β = -0.262, P = 0.008), BMI (β = -0.243, P = 0.004) and T2D duration (β = -0.277, P = 0.005), remained significantly associated with CTRP9 [Table 3].

On performing simple linear regression using MCP-1 as the dependent variable and other biochemical parameters as the independent variables, MCP-1was found to be significantly