ORIGINAL ARTICLE

Correlation between Highly Sensitive C Reactive Protein and Development of Microvascular Complications of Type 2 Diabetes Mellitus

[1]Amal Hassaan, [2]Mohamed I. Abd El-Ghany, [1]Waleed Eldars*, [1]Talat A. Othman
[1]Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2]Department of Internal medicine, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Key words:
T2DM, microvascular complications, hsCRP.

Corresponding Author:
Waleed Eldars
Department of Medical Microbiology & Immunology, Faculty of Medicine, Mansoura University, Egypt.
Tel: 020-1061494956
wellydars@gmail.com

ABSTRACT

Background: *Microvascular problems associated with type 2 diabetes mellitus (T2DM) are a leading source of morbidity and mortality in T2DM patients, thus the search for new biomarkers to identify and treat those at high risk is intensifying.* ***Objective:*** *This controlled cross-sectional study aimed to investigate whether there is a correlation between serum levels of highly sensitive C reactive protein (hsCRP) and development of microvascular complications in T2DM.* ***Methodology:*** *Hundred and thirty-five subjects aged from 35 to 70 years were subdivided into 3 groups. Detailed clinical history was taken. Serum levels of hsCRP were estimated using Immune Turbidimetry.* ***Results:*** *Serum hsCRP levels were higher in group A than other studied groups. However, there was no statistically significant difference between group A and B(P=0.35). Serum hsCRP levels were significantly higher in patients with diabetic nephropathy than patients without nephropathy (P=0.016). The difference between serum hsCRP levels in patients with and without retinopathy and neuropathy was not significant (P=0.39 and P=0.53 respectively).* ***Conclusion:*** *Serum hsCRP can be used as a novel marker for diagnosis of microvascular complications in T2DM patients.*

Abbreviations: (T2DM: Type 2 Diabetes Mellitus, hsCRP: highly sensitive C Reactive Protein, eGFR: Estimated Glomerular filteration Rate, BUN: Blood Urea Nitrogen, HbA1c: Glycosylated Hemoglobin).

INTRODUCTION

Diabetes is linked to both microvascular and macrovascular complications, such as retinopathy, nephropathy, and neuropathy (microvascular) and ischemic heart disease, peripheral vascular disease, and cerebrovascular disease (macrovascular), resulting in organ and tissue damage in one-third to one-half of diabetics[1]. Macro and microvascular problems are a leading cause of morbidity and mortality in T2DM patients, thus the search for new biomarkers to identify and treat those at high risk is intensifying [2].

The liver produces C-reactive protein (CRP), which is a pentameric acute phase reactant. Interleukin-6 is the main regulator of its production. With infection, trauma, surgery, and other acute inflammatory events, serum CRP levels can rise by up to 1000-fold. Chronic inflammatory conditions, such as autoimmune diseases and cancer, can cause permanent elevations in CRP levels in the blood[3]. The high-sensitivity C-reactive protein (hsCRP) assay is more sensitive than the traditional C-reactive protein (CRP) assay. There have been multiple studies showing type 2 diabetics had higher hsCRP levels in their blood than non-diabetic people [4].

Hemoglobin A1c levels are linked to hsCRP levels and future cardiovascular risk in people with type 1 and type 2 diabetes. Serum hsCRP levels also rise as the stage of -cell dysfunction and insulin resistance progress[5].

We need to further investigate the relation between serum level of hsCRP and development of microvascular complications in T2DM patients. This may help to early identify and control these complications.

The aim of this work is to investigate whether there is a correlation between serum hsCRP levels and development of microvascular complications in T2DM.

METHODOLOGY

Study population:

The participants in this controlled cross-sectional study range in age from 35 to 70 years old and comprise 100 people with type 2 diabetes and 35 people without diabetes. Then the participants were classified into 3 groups: Group A: 55 subjects with type 2 diabetes with complications (30 women & 25 men); Group B: 45 subjects with type 2 diabetes without complications (25 women & 20 men) and Group C: 35 normal healthy

subjects (20 women & 15 men). All cases and controls, including men and women, were recruited from the Mansoura specialized internal hospital's diabetes and endocrinology outpatient clinic. After receiving approval from Mansoura Faculty of Medicine institutional research board, the study was conducted from December 2019 to November 2020. (MFM-IRB). The local ethical committee at the Mansoura college of medicine authorized the participants' informed written consent to participate in the study. The diabetic state was confirmed or excluded according to the revised American Diabetes Association criteria. The followings were excluded, subjects with Type 1 DM, subjects without diabetes but with CAD, pregnant women, subjects with infection and malignant disorders.

Clinical and Anthropometric Measurements:

All participants were given a complete medical history, anthropometric measurements (weight, height, and BMI), and a thorough clinical examination, with a focus on any microvascular problems. Using a fundus camera and retinal imaging, subjects with type 2 diabetes were assessed for various microvascular problems such as diabetic retinopathy (TRC-50DX Series). The usual diagnosis of diabetic nephropathy was based on the quantitative urine albumin/creatinine ratio in morning spot urine samples, eGFR, elevated BUN, and creatinine. Based on the clinical evidence available, diabetic neuropathy was diagnosed.

Collection and processing of samples:

Venous blood samples (2 ml) were withdrawn from each subject via proper venipuncture technique under complete aseptic condition. Samples were transported to Immunology Unit, Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, for further processing. Blood samples were centrifuged for 20 minutes at a velocity of 2000-3000 R.P.M. for separation of serum. Serum levels of hsCRP were measured using Immune Turbidimetry (BioSystems). HbA1c was measured by the ion-exchange chromatography method (Biosystem co, Spain). Plasma glucose was measured by the glucose oxidase method (Cobas Integra 400 plus, Germany).

Statistical Analysis:

It was carried out via the Statistical Package for Social Science (SPSS) version 17 program on windows 7. The Kolmogorov-Smirnov test was hired to check the normality of quantitative data. Qualitative data were denoted in the form of numbers and percentages, while quantitative data were denoted in the form of mean ± standard deviation (mean± SD). χ 2 and Student's t-tests were used to compare variables. Pearson's correlation coefficient test was used to detect the correlation between variables. Results with a p-value of less than or equal to 0.05 were considered statistically significant. Receiver Operating characteristic Curve (ROC) Analysis was computed to identify serum hsCRP cutoff values differentiating between people with microvascular complications and people with diabetes only.

RESULTS

This controlled cross-sectional study was conducted over a period of 12 months from December 2019 till November 2020, on patients attending to the diabetic outpatient clinic at Mansoura specialized internal Hospital. During this period, 135 subjects were enrolled in the study; 100 were type 2 diabetic patients and 35 were non diabetics.

Demographic data of subjects:

Our participants were classified into three groups: group (A) included 55 (25 men, 30 women) subjects with type 2 diabetes who have shown microvascular complications; group B included 45 subjects with type 2 diabetes but without microvascular complications; and group C which included age and gender-matched healthy subjects (n=35: 15 men and 20 women).

The medians of age were 48 (37-56), 46 (37-56), and 45(36-56), respectively. Also, the medians of BMIs of the three groups were 32.4 (29.9-34.3), 32.1 (29.8-34.4), and 31.2 (29.6-34.2), in order.

Baseline lab findings and serum hsCRP levels of the studied groups:

All baseline lab findings were significantly higher in group A and group B compared to group C ($P3<0.001$, $P4<0.001$) except HDL which was significantly lower in group A and group B compared to group C ($P3<0.001$, $P4<0.001$).

Fasting blood glucose, HbA1c, LDL and Albumin/ creatinine ratio were significantly higher among group A participants compared to group B. Regarding cholesterol, triglycerides and HDL, the difference was not statistically significant between group A and group B.

Serum hsCRP levels were higher in group A than other studied groups. However, there was no statistically significant difference between group A and B ($P2=0.35$), as shown in table (1).

Table 1: Baseline lab findings and serum hsCRP levels of the studied groups

	Group A	Group B	Group c	P value
FBG (mg/dl) (Mean ± SD)	197.7±31.16	140.5±15.5	90.2±6.82	*P*1<0.001 *P*2<0.001 *P*3<0.001 *P*4<0.001
HbA1c (%) (Mean ± SD)	10.4±1.49	7.74±0.44	4.42±0.45	*P*1<0.001 *P*2<0.001 *P*3<0.001 *P*4<0.001
Cholesterol(mg/dl) (Mean ± SD)	231.8±38.5	221.5±32	162.4±26.9	*P*1<0.001 *P*2=0.13 *P*3<0.001 *P*4<0.001
Triglycerides Median (IQR)	230 (177-260)	200 (165-240)	100 (85-112)	*P*1<0.001 *P*2=0.12 *P*3<0.001 *P*4<0.001
LDL (mg/dl) Median (IQR)	144 (134-168)	137 (124-162)	92 (78-113)	*P*1<0.001 *P*2=0.04 *P*3<0.001 *P*4<0.001
HDL (mg/dl) Median (IQR)	38 (35-42)	40 (35-41.5)	47 (45-50)	*P*1<0.001 *P*2=0.35 *P*3<0.001 *P*4<0.001
Albumin/ creatinine ratio Median (IQR)	25 (18-395)	16 (12.5-18)	9 (7-10)	*P*1<0.001 *P*2<0.001 *P*3<0.001 *P*4<0.001
hsCRP(mg/l) Median (IQR)	10.6 (2.3-13.6)	4.2 (2.7-7.6)	2.6 (2.1-2.9)	*P*1<0.001 *P*2=0.35 *P*3<0.001 *P*4=0.001

*Significant $P \leq 0.05$. *P*1 Group A vs. Group B vs. Group C, *P*2 Group A vs. Group B, *P*3 Group B vs. Group C, *P*4 Group A vs. Group C. FBG: fasting blood glucose, HbA1c: glycosylated hemoglobin, LDL: low density lipoproteins, HDL: high density lipoproteins, hsCRP: highly sensitive c reactive protein.

Serum hsCRP levels in relation to presence or absence of diabetic microvascular complications:

Serum hsCRP levels were significantly higher in patients with diabetic nephropathy than patients without nephropathy (*P*=0.016). However, the difference between serum hsCRP levels in patients with and without retinopathy and neuropathy was not significant as shown in table (2).

Table 2: Serum hsCRP levels in relation to presence or absence of diabetic microvascular complications.

	Diabetic Retinopathy		P value
	Yes (n=40)	No (n=60)	
hsCRP (mg/l)	5.5 (2.2-14)	4 (2.6-8.3)	*P*=0.39
	Diabetic Neuropathy		P value
	Yes (n=55)	No (n=45)	
hsCRP (mg/l)	4.9 (2.3-13.6)	4.2 (2.7-7.6)	*P*=0.53
	Diabetic Nephropathy		P value
	Yes (n=20)	No (n=80)	
hsCRP (mg/l)	12 (2.8-21)	3.9 (2.4-8.3)	*P*=0.016

DISCUSSION

The serum levels of high sensitivity C-reactive protein (hsCRP), which is a marker of systemic inflammation and a mediator of atherosclerotic disease, have been correlated with the risk of cardiovascular disease and type 2 diabetes mellitus [6].

Our controlled cross-sectional study was conducted over a period of 12 months from December 2019 till November 2020, on patients attending to the diabetic outpatient clinic at Mansoura Specialized Internal Hospital. Our study aimed to investigate whether there is a correlation between sTREM2 and hsCRP and development of microvascular complications in T2DM. Our study was conducted on 135 subjects who were further divided into 3 groups; group A included 55 diabetic patients with microvascular complications, group B included 45 diabetic patients without complications and group C included 35 non-diabetic subjects.

Our study showed that hsCRP levels were non-significantly higher in diabetic patients with microvascular complications than diabetic patients without complications (P=0.35). This finding agrees with that detected by Aslam and Chandrasekhara[7] who found a significant positive correlation between hsCRP levels and microvascular complications (P<0.0001).

Our study showed that serum hsCRP levels were significantly higher in patients with diabetic nephropathy than patients without nephropathy (P=0.016). However, the difference between hsCRP levels in patients with and without retinopathy and neuropathy was not significant. This finding matches with the result of Kang et al.[6] who reported that there were no differences between the mean serum hsCRP levels of those with and without retinopathy (P = 0.447). There were no differences between the mean serum hsCRP levels of those with and without neuropathy (P = 0.398). There was a significant difference in the serum hsCRP levels between the patients with nephropathy and those without nephropathy (P = 0.003). The same result was obtained by Najafi et al.[8]. On the same way, Varma et al.[9] found that patients with diabetic nephropathy have significant higher hsCRP than diabetic patients without nephropathy (P<0.001). Also, Hayashino et al.[10] demonstrated that elevated levels of hsCRP were associated with the development of nephropathy (P<0.001) but not associated with the progression of nephropathy (P=0.575). Furthermore, Del Canizo Gómez et al.[11] found that urinary albumin excretion levels higher than 12 mg/24 h, hsCRP >3 mg/L and hypertension were all independent risk factors for development of microvascular complications (nephropathy and /or retinopathy) in patients with T2DM.

Higher hsCRP in diabetic nephropathy can be hypothesized that low-grade inflammation in diabetes might stimulate endothelial cells and podocytes towards accumulation of extracellular matrix in the glomerular basement membrane. Alternatively, the deposition of extracellular matrix in excess may induce an acute-phase reaction [12].

There are certain limitations to our research. Because of the study's cross-sectional design, it's difficult to determine if serum hsCRP levels are linked to the development of diabetic microvascular problems. To explain the underlying mechanisms, more longitudinal prospective studies with a bigger sample size are needed. In addition, other components of obesity-related and inflammatory factors, such as interleukins and tumour necrosis factors, were not available in the current study, and this information could be useful in gaining a better understanding of the link between hsCRP levels and diabetic microvascular complications.

It was concluded that hsCRP can be used as a novel marker for diagnosis of microvascular complications in T2DM patients.

Ethics Approval and Consent To Participate

The study protocol was approved by the Ethics Review Board of Faculty of Medicine, Mansoura University, Egypt.

Human and Animal Rights

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

Consent for Publication

Informed written consent was obtained from all participants.

Standards of Reporting

This study was conducted in accordance with the STROBE guidelines.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

ACKNOWLEDGEMENTS

Dr Nahed Abdallah

Mr Saeed Gamal

REFERENCES

1. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Physical therapy. 2008 Nov 1; 88(11):1322-1335.
2. Barutta F, Bruno G, Matullo G, Chaturvedi N, Grimaldi S, Schalkwijk C, Stehouwer CD, Fuller JH, Gruden G. MicroRNA-126 and micro-/macrovascular complications of type 1 diabetes in the EURODIAB Prospective Complications Study. Acta diabetologica. 2017 Feb 1; 54(2):133-139.
3. Yu H, Huang Y, Chen X, Nie W, Wang Y, Jiao Y, Reed GL, Gu W, Chen H. High-sensitivity C-reactive protein in stroke patients–The importance in consideration of influence of multiple factors in the predictability for disease severity and death. Journal of Clinical Neuroscience. 2017 Feb 1; 36:12-19.
4. Kang ES, Kim HJ, Kim YM, Lee S, Cha BS, Lim SK, Kim HJ, Lee HC. Serum high sensitivity C-reactive protein is associated with carotid intima-media thickness in type 2 diabetes. Diabetes research and clinical practice. 2004 Dec 1; 66: S115-S120.
5. Pfützner A, Forst T. High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. Diabetes technology & therapeutics. 2006 Feb 1; 8(1): 28-36.
6. Kang ES, Kim HJ, Ahn CW, Park CW, Cha BS, Lim SK, Kim KR, Lee HC. Relationship of serum high sensitivity C-reactive protein to metabolic syndrome and microvascular complications in type 2 diabetes. Diabetes research and clinical practice. 2005 Aug 1; 69(2):151-159.
7. Aslam M, Chandrasekhara P. Correlation of Fibrinogen and HsCRP with Microvascular Complications of Type 2 Diabetes Mellitus. IJHSR. 2016; 6(5):25-32.
8. Najafi L, Malek M, Valojerdi AE, Khamseh ME. Acute phase proteins and diabetes microvascular complications. International Journal of Diabetes in Developing Countries. 2016 Mar 1;36(1):10-17.
9. Varma V, Varma M, Varma A, Kumar R, Bharosay A, Vyas S. Serum total sialic acid and highly sensitive C-reactive protein: Prognostic markers for the diabetic nephropathy. Journal of laboratory physicians. 2016 Jan;8(01):025-029.
10. Hayashino Y, Mashitani T, Tsujii S, Ishii H. Serum high-sensitivity C-reactive protein levels are associated with high risk of development, not progression, of diabetic nephropathy among Japanese type 2 diabetic patients: a prospective cohort study (Diabetes Distress and Care Registry at Tenri [DDCRT7]). Diabetes Care. 2014 Nov 1; 37(11):2947-2952.
11. del Canizo Gómez FJ, Pérez CF, Ruiz IM, de Gorospe Pérez-Jáuregui C, Rodríguez BS, Losada TG, Galindo AS. Microvascular complications and risk factors in patients with type 2 diabetes. Endocrinología y Nutrición. 2011 Apr 1; 58(4):163-168.
12. Dalla Vestra M, Mussap M, Gallina P, Bruseghin M, Cernigoi AM, Saller A, Plebani M, Fioretto P. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. Journal of the American Society of Nephrology. 2005 Mar 1;16(3 suppl 1): S78-S82.