

## Study of high-sensitivity C-Reactive protein levels in subjects with type 2 diabetes mellitus

K. Prathibha Bharathi<sup>1</sup>, Shivakumar<sup>2</sup>, M. Krishnamma<sup>3</sup>, J.N. Naidu, M. Prasad<sup>4\*</sup>

<sup>1</sup>Chief Consultant, <sup>2</sup>Assistant Professor, <sup>3</sup>Professor, <sup>4</sup>Professor & HOD, <sup>5</sup>Tutor, Dept. of Biochemistry, <sup>1</sup>Apollo Speciality & Hospital, Nellore, Andhra Pradesh, <sup>2</sup>Sri Siddhartha Medical College & Hospital, Tumkur, Karnataka, <sup>3,4,5</sup>Narayana Medical College & Hospital, Nellore, Andhra Pradesh, India

**\*Corresponding Author:**

Email: m.prasadnaidu@ymail.com

### Abstract

**Introduction:** The Diabetes mellitus was characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The two broad categories of diabetes are type 1 and type 2. Type 2 Diabetes Mellitus (T2DM) is the most common form of diabetes. The role of elevated high sensitivity C-reactive protein (hs-CRP) as a risk marker for cardiovascular diseases, including coronary heart disease, stroke and peripheral arterial disease is well established through consistent results from a number of prospective studies. More recent data suggest that hs-CRP is superior to other markers of inflammation for risk evaluation.

**Materials and Methods:** The present study was conducted over a period of one year on outpatients attending the General Medicine Department at Narayana General Hospital, Nellore. The study was undertaken on 50 type 2 Diabetes mellitus and 50 normal healthy controls. Both male and females in the age group of 35 – 70 years were included.

**Results:** Fasting blood glucose, post prandial blood glucose, and hs-CRP levels was measured in 50 T2DM cases and 50 age matched healthy controls. The mean and standard deviation were calculated for all the Biochemical parameters. The significance between the groups were determined using Student t- test for Equality of means. The *p*-value of < 0.05 was considered significant.

**Conclusion:** This study concludes that there is increase in hs-CRP levels in T2DM cases compared with controls. hs-CRP is an inflammatory marker and has role in atherosclerosis. From this study it is observed that there is moderate correlation between hs-CRP levels and it increases the risk of atherosclerosis.

**Keywords:** Diabetes Mellitus, hs-CRP, Hyperglycemia, Inflammation.

**Received:** 04<sup>th</sup> October, 2017

**Accepted:** 17<sup>th</sup> November, 2017

### Introduction

Diabetes mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The two broad categories of diabetes are type 1 and type 2.<sup>1</sup> Type 2 diabetes is the most common form of diabetes. Type 2 diabetes is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production.<sup>2</sup> The prevalence of diabetes, constituted chiefly by type 2 diabetes mellitus, is a global public health threat. The prevalence among adults aged 20-70 years is expected to rise from 285 million in 2010 to 438 million by the year 2030.<sup>3</sup> The present trend indicates that more than 60% of the world's diabetic population will be in Asia.<sup>4</sup> The metabolic disturbance associated with diabetes causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous

burden on the individual with diabetes and on the health care system.<sup>5</sup> The risk of death from cardiovascular disease (CVD) is two to six times greater in people with type 2 diabetes than those without diabetes and is the leading cause of morbidity and mortality in type 2 diabetes. At least 50% of deaths are caused by coronary heart disease (CHD).<sup>6</sup> Coronary artery disease (CAD) is a major vascular complication of diabetes mellitus and reveals high mortality. Up to 30% of diabetic patients with myocardial ischemia remain asymptomatic and are associated with worse prognosis compared to non-diabetic counterpart, which warrants routine screening for CAD in diabetic population.<sup>7</sup> Epidemiological studies indicate that diabetes mellitus can accelerate atherosclerotic processes and increase the incidence of cardiovascular events and strokes.<sup>8</sup>

The role of elevated hs-CRP as a risk marker for cardiovascular diseases, including coronary heart disease, stroke and peripheral arterial disease is well established through consistent

results from a number of prospective studies. More recent data suggest that hs-CRP is superior to other markers of inflammation for risk evaluation.<sup>9</sup>

The hs-CRP is the measurement of CRP level with greater accuracy. The lower limit of its measurement is 0.01 mg/L and the measurement is more than 100 times sensitive compared to usual CRP measurement (lower limit 5 mg/L).<sup>10</sup>

In 1930, Tillett and Francis discovered CRP while working on patients with pneumococcal infection. A precipitate was observed when serum from these patients is mixed with C-polysaccharide coat of streptococcus pneumonia. CRP is the first acute phase protein to be described and is highly sensitive systemic marker of inflammation and tissue damage.<sup>11</sup>

C-Reactive protein is a non-glycosylated polymeric protein consisting of 5 identical subunits, non covalently linked to form a disc shaped cyclic polymer with a molecular weight 1, 15, 000 to 1, 40, 000. The protein contains little or no carbohydrate.<sup>12</sup>

The structure of CRP contains a calcium binding loop from one protomer and coordinates into the calcium site of a second protomer to form the pentameric structure. The protomers are noncovalently associated in an annular configuration with cyclic pentameric symmetry. Each protomer has the characteristic lectin fold composed of a two layered  $\beta$ -sheet. The ligand binding site is composed of loops with two calcium ions bound 4 Å apart by protein side chains, which is located on the concave phase. Other phase carries a single alpha-helix. CRP is stable and has a half-life of approximately 19 hours.<sup>13</sup>

### **Production of CRP**

Hepatocytes produce CRP in response to tissue injury or infection. CRP is also produced in kidneys, neurons and bowels to a lesser extent. Loss of Pentameric symmetry of CRP results in a modified or monomeric CRP which may be the major CRP isoform promoting the proinflammatory response in coronary arteries.<sup>14</sup>

### **Role of hs-CRP in Atherogenesis**

hs-CRP has been linked to the process of atherogenesis in all stages from participating in endothelial dysfunction, atherosclerotic plaque formation, plaque maturation, plaque destabilization and eventual rupture. Endothelial cell dysfunction and progression of atherosclerosis is by decreasing nitric oxide synthesis. CRP is present in the vessel wall, where it induces expression of the adhesion molecules E-selectin, VCAM-1 and ICAM-1 by

endothelial cells, and serves as a chemoattractant for monocytes as mediated by induction of MCP-1.<sup>15</sup> CRP opsonizes LDL and facilitates native LDL entry into macrophages. CRP binds to plasma membranes of damaged cells and activates complement via the classical pathway leading to maturation of atherosclerotic lesions. Damaged endothelial cells release platelet derived growth factor (PDGF) which causes proliferation of smooth muscle cells and migration from the media to the intimal layer of the arterial wall. This contributes to the formation of atherosclerotic plaques.<sup>16</sup>

Laboratory and experimental evidence indicate that atherosclerosis is not only a disease of lipid accumulation but also represents a chronic inflammatory process. Researchers have hypothesized that inflammatory marker such as hs-CRP may provide an adjunctive method for global assessment of cardiovascular risk. In support of this hypothesis, several large-scale prospective epidemiological studies have shown that plasma levels of hs-CRP are a strong independent predictor of risk of future myocardial infarction, stroke, peripheral arterial disease, and vascular death among individuals without known cardiovascular disease.

### **Materials and Methods**

The present study was conducted over a period of one year on outpatients attending the General Medicine Department at Narayana General Hospital, Nellore. The study was undertaken on 50 type 2 Diabetes mellitus and 50 normal healthy controls. All of them were in the age group of 35-70 years. Both sexes included. Institutional ethical committee of Narayana Medical College and Hospital, Nellore, Andhra Pradesh has approved the study and written informed consent obtained from the patients. This study includes diabetic for at least one year duration and normal hepatic function and without complications of neuropathy, retinopathy, overt nephropathy, coronary artery diseases. The present study excludes patients with thyroid stimulating drugs, corticosteroids, lipid-lowering drugs, oral contraceptives, aspirin, sulphonamides and pregnant women.

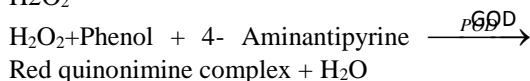
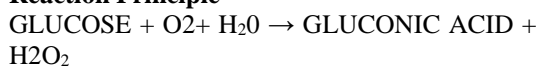
### **Specimen collection**

Subjects under this study were advised to fast over night (twelve hours). Blood samples were collected in fasting condition .5ml venous blood was collected from the each subjects and it was transferred to the Plain tube and serum is separated by centrifugation and stored at -20°C for measured. Haemolysed and lipemic samples

are avoided. For adequate quality control both normal, abnormal reference control serum solutions and calibrators were run before each testing. Other factors influencing the quality like proper functioning of instrument, glassware, cuvettes and distilled water were taken care.

Estimation Of Plasma Glucose by the Glucose Oxidase and Peroxidase (GOD POD) method using a commercially available kit Human (gmbh Germany) using Humastar 300 chemistry analyzer (Human gmbh Germany). Enzymatic colorimetric test for glucose method without deproteinisation. Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. Hydrogen peroxide formed in catalysed by peroxidase to release nascent oxygen. Oxygen is turn for reaction.

### Reaction Principle



### Performance characteristics

The test is linear up to glucose conc. of 400 mg /dl or 22.2mmol/l. If the glucose concentration of sample is over this limit, sample is diluted among distilled water and the test is repeated. The result is multiplied by 3.

Estimation Of hs-CRP by turbidometric immunoassay using commercially available kit (Erba) and Humastar 300 chemistry analyzer (Human gmbh Germany). The CRP-ultrasensitive is a quantitative turbidimetric test for the measurement of low levels of CRP in human serum or plasma. Latex particles coated with specific anti- human CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change,

dependent upon the CRP contents of the patient sample that can be quantified, by comparison from a calibrator of known CRP concentration.

### Calibration

Use CRP Ultra Calibrator Reference 43035. The sensitivity of the assay and the target value of the calibrator have been standardized against the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM). CRP Calibrator; Reconstitute with 2.0 ml of distilled water. Mix gently and bring to room temperature for about 10 minutes before use. Calibration curve; Prepare the following CRP calibrator dilutions in NaCl 9g/L. Multiply the concentration of the CRP calibrator by the corresponding factor stated in table 1 to obtain the CRP concentration of each dilution.

### Storage and stability

Specimen; Fresh serum. Stable 7 days at 2-8°C or 3 months at -20°C. The samples with particles or fibrin should be centrifuged before testing. Do not use hemolyzed or lipemic samples. Reference was below 3 mg/L is considered normal.

### Results

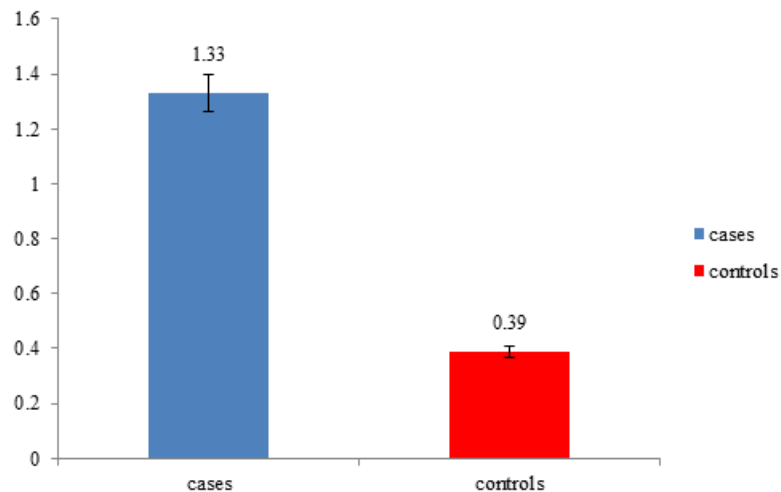
Fasting blood glucose, post prandial blood glucose, and hs-CRP levels measured in 50 T2DM cases and 50 age matched healthy controls. The mean and standard deviation were calculated for all the Biochemical parameters. The significance between the groups were determined using Student t- test for Equality of means. The p-value of < 0.05 was considered significant.

**Table 1: High-sensitivity C-Reactive protein levels in cases and controls**

S. No.	Parameter	Cases	Controls		P value	t value
		Mean ± S.D	Mean	S.D.		
1.	hs-CRP (mg/dl)	1.33 ± 1.12	0.70	0.39	<0.0001	7.512

**P value:** Significant

**Graph 1: Mean hs-CRP levels in cases and controls**

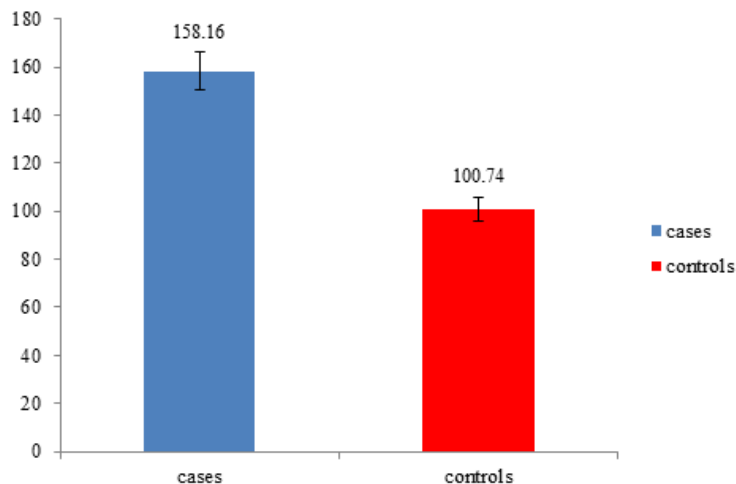


**Table 2: Fasting blood glucose levels in patients and controls**

S. No.	Parameter	Cases	Controls		P value	t value
		Mean ± S.D.	Mean	S.D.		
1.	FBS (mg/dl)	158.16 ± 75.1	100.74	21.64	0.0001	5.197

**P value:** Significant

**Graph 2: Mean FBS levels in cases and controls**

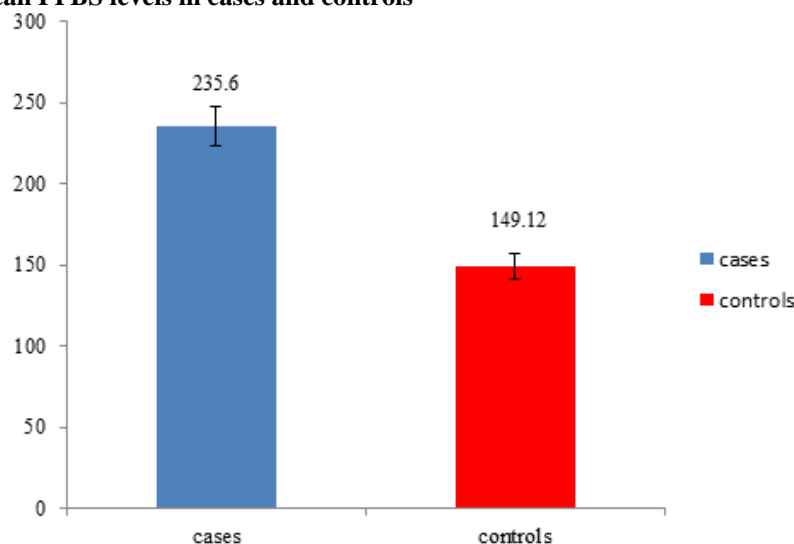


**Table 3: Post prandial blood glucose levels in patients and controls**

S. No.	Parameter	Cases	Controls		P value	t value
		Mean ± S.D.	Mean	S.D.		
1.	PPBS (mg/dl)	235.6 ± 96.55	149.12	19.17	0.0001	6.21

**P value:** Significant

**Graph 3: Mean PPBS levels in cases and controls**



**Table 4: MEAN+SD AND P, t value in t2DM and controls**

S. No.	Parameter	Cases	Controls	P value	t value
		Mean± S.D.	Mean± S.D.		
1.	hs-CRP	1.33±1.12	0.070±0.39	0.0001	7.512
2	FBS	158.16±75.1	100.74±21.64	0.0001	5.197
3.	PPBS	235.6±96.55	149.12±19.17	0.0001	6.21

## Discussion

Diabetes mellitus is a metabolic disorder. The metabolic disturbance associated with long standing DM causes secondary pathophysiologic changes in multiple organ systems leading to various life threatening complications like atherosclerosis, retinopathy, neuropathy, nephropathy. The prevalence of T2DM is increasing alarmingly worldwide. Coronary artery atherosclerosis is the major cause of mortality among diabetes population. Diabetes mellitus can accelerate atherosclerotic processes. The study is aimed at “evaluating the hs-CRP levels in type 2 diabetes mellitus patients”. The present study included 100 subjects. Among them 50 were type 2 diabetes cases and 50 were healthy controls. In both cases and controls high-Sensitivity C-Reactive Protein levels measured.

In this study mean and standard deviation of hs-CRP in cases were  $1.332 \pm 1.12$  and in controls were  $0.707 \pm 0.39$ . This increase was statistically significant ( $P=0.0003$ ). Increasing evidence suggests that hs-CRP may be directly

involved in atherothrombogenesis that extends beyond its previously accepted role as an inflammatory marker. This hs-CRP is present in the vessel wall, where it induces expression of the adhesion molecules Eselectin, VCAM-1 and ICAM-1 by endothelial cells, and serves as a chemoattractant for monocytes as mediated by induction of MCP-1. CRP opsonizes LDL and facilitates native LDL entry into macrophages. CRP binds to plasma membranes of damaged cells and activates complement via the classical pathway for maturation of atherosclerotic lesions. CRP is associated with endothelial cell dysfunction and progression of atherosclerosis, possibly by decreasing nitric oxide synthesis. The results were in accordance with the study of Giovanna castoldi et al., (2007),<sup>17</sup> Li Jin Pu, Lin Lu et al.,(2006).<sup>18</sup>

Pfützner A, et al<sup>19</sup> shows efficacy of different anti-diabetes treatments on a variety of cardiovascular risk markers. Intensive insulin therapy may be decreases the inflammation, although these effect may influenced through a degree of weight gain. Treatment within

peroxisome proliferator-activated receptor  $\gamma$  has lead directed towards substantial decreased of hsCRP along with further cardiovascular risk markers in different comparator studies. Considering for these outcome is showed to be independent of this degree of glycemic development, it could be considering as a class specific effect. Even if findings translate into a decreases of total cardiovascular mortality will soon be shown through the currently running thiazolidinedione further studies. Positive results in this study have further strengthened the usefulness of hs-CRP as a predictive laboratory marker for cardiovascular disease risk in patients with the T2DM.

Devaraj S, Jialal I. et al<sup>20</sup> reporting following 3 months of supplementation and following a 2 month washout grade. DM2-MV subjects have been increased hs-CRP and monocyte IL-6 compared to controls. Alpha tocopherol (AT) supplementation was shown significantly to reduced levels of C-reactive protein and monocyte interleukin-6 among this group. In conclusion, AT therapy reduced inflammation in T2DM patients and this can be an adjunct therapy in the prevention of atherosclerosis.

### Conclusion

The risk of death from cardiovascular disease is from two to six times greater in people with type 2 diabetes than those without diabetes and is the leading cause of morbidity and mortality in type 2 diabetes. At least 50% of deaths are caused by coronary heart disease. Previous studies have shown that up to 30% of the diabetic patients with CAD had silent ischemia and experienced poor outcome following acute coronary events, indicating clinical importance of screening asymptomatic CAD in diabetic population. From this study, I conclude that measuring hs-CRP levels are useful for early detection of cardiovascular risk before the inflammation sets in.

### Summary

The Coronary artery atherosclerosis is the major cause of morbidity and mortality among diabetes population. Diabetes mellitus can accelerate atherosclerotic processes. The present study was conducted over a period of one year on outpatients attending the General Medicine department of Narayana General Hospital, Nellore. In this study I found that significant increase in hs-CRP levels in T2DM cases compared with controls. hs-CRP is an inflammatory marker and has role in

atherosclerosis. From this study we understand that there is inverse relation hs-CRP levels may increase the risk for atherosclerosis.

**Conflict of interest:** None declared

**Acknowledgements:** Cooperation with Dept of Biochemistry, Narayana Medical College and Hospital, Chinthareddy palem, Nellore, 524003, Andhra Pradesh, India.

### References

1. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nature reviews. Immunology*. 2011 Feb 1;11(2):98.
2. Bell GI, Polonsky KS. Diabetes mellitus and genetically programmed defects in  $\beta$ -cell function. *Nature*. 2001 Dec 13;414(6865):788-91.
3. Ramachandran A, Snehalatha C, Shetty AS, Nanditha A. Trends in prevalence of diabetes in Asian countries. *World journal of diabetes*. 2012 Jun 15;3(6):110.
4. Ramachandran A, Snehalatha C, Ma RC. Diabetes in South-East Asia: an update. *Diabetes research and clinical practice*. 2014 Feb 28;103(2):231-7.
5. Janani C, Kumari BR. PPAR gamma gene—a review. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2015 Mar 31;9(1):46-50.
6. Asia Pacific Cohort Studies Collaboration. The effects of diabetes on the risks of major cardiovascular diseases and death in the Asia-Pacific region. *Diabetes care*. 2003 Feb 1;26(2):360-6.
7. Valensi P, Lorgis L, Cottin Y. Prevalence, incidence, predictive factors and prognosis of silent myocardial infarction: a review of the literature. *Archives of cardiovascular diseases*. 2011 Mar 31;104(3):178-88.
8. Daniels SR, Greer FR. Lipid screening and cardiovascular health in childhood. *Pediatrics*. 2008 Jul 1;122(1):198-208.
9. Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. *Clinical chemistry*. 2001 Mar 1;47(3):403-11.
10. Roberts WL, Sedrick R, Moulton L, Spencer A, Rifai N. Evaluation of four automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. *Clinical Chemistry*. 2000 Apr 1;46(4):461-8.
11. Bayne CJ, Gerwick L. The acute phase response and innate immunity of fish. *Developmental & Comparative Immunology*. 2001 Oct 12;25(8):725-43.
12. Omtvedt LA, Wien TN, Myran T, Sletten K, Husby G. Serum amyloid P component in mink, a non-glycosylated protein with affinity for phosphorylethanolamine and phosphorylcholine. *Amyloid*. 2004 Jun 1;11(2):101-8.

13. Murray LA, inventor; Promedior, Inc., assignee. Treatment methods for hypersensitive disorders. United States patent US 9,233,140. 2016 Jan 12.
14. Sjowall C, Bengtsson T, Skogh T. CRP and anti-CRP autoantibodies in systemic lupus erythematosus. *Current Rheumatology Reviews*. 2005 Jan 1;1(1):81-9.
15. Botvinick EH, Perini R, Bural G, Chen W, Chryssikos T, Houseni M, Hernandez-Pampaloni M, Torigian DA, Alavi A. The aging of the heart and blood vessels. *Nuclear medicine* 2007 Mar 31 (Vol. 37, No. 2, pp. 120-143). WB Saunders.
16. Moalli F, Jaillon S, Inforzato A, Sironi M, Bottazzi B, Mantovani A, Garlanda C. Pathogen recognition by the long pentraxin PTX3. *BioMed Research International*. 2011 Jun 2;2011.
17. Castoldi G, Galimberti S, Riva C, Papagna R, Querci F, Casati M, Zerbini G, Caccianiga G, Ferrarese C, Baldoni M, Valsecchi MG. Association between serum values of C-reactive protein and cytokine production in whole blood of patients with type 2 diabetes. *Clinical science*. 2007 Jul 1;113(2):103-8.
18. Pu LJ, Lu L, Xu XW, Zhang RY, Zhang Q, Zhang JS, Hu J, Yang ZK, Ding FH, Chen QJ, Lou S. Value of serum glycated albumin and high-sensitivity C-reactive protein levels in the prediction of presence of coronary artery disease in patients with type 2 diabetes. *Cardiovascular Diabetology*. 2006 Dec 20;5(1):27.
19. 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.
20. Devaraj S, Jialal I. Alpha tocopherol supplementation decreases serum C-reactive protein and monocyte interleukin-6 levels in normal volunteers and type 2 diabetic patients. *Free Radical Biology and Medicine*. 2000 Oct 15;29(8):790-2.