High-sensitivity C-reactive protein, Malondialdehyde and their association with Glycated hemoglobin (HbA1c) in type 2 diabetes patients

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A B S T R A C T

Introduction: Type 2 diabetes mellitus is a major public health problem worldwide and accompanied by enduring vascular complications, which leads to morbidity and mortality. Inflammation plays a major role in the pathogenesis of type 2 diabetes mellitus. High sensitivity C-reactive protein is an acute phase protein synthesized by the liver and has been revealed as sensitive, systemic inflammatory marker. Oxidative stress, low grade systemic inflammation contributes to insulin resistance and is linked to the characteristics of metabolic syndrome and type 2 diabetes mellitus.

Objectives: The present study was to evaluate hs-CRP, malondialdehyde (MDA) levels in type 2 diabetic patients compared with healthy controls and correlate these levels with glycated hemoglobin (HbA1C) and insulin resistance.

Materials and Methods: Fifty type 2 diabetic patients with age group of 35 to 45 years were selected for this study and 50 age matched healthy subjects were selected as controls. Serum hs-CRP and insulin was assessed by ELISA, malondialdehyde (MDA) was assessed by Thiobarbituric Acid Reactive Substances (TBARS) method and other routine investigations were carried out by standardized protocols with ERBA EM-360 fully automated analyzer.

Results: The mean serum hs-CRP and MDA levels were significantly increased in type 2 diabetic patients compared with healthy controls. Hs-CRP and MDA levels were shown significant positive correlation with glycated hemoglobin (HbA1C), insulin resistance, triglycerides and negative correlation with HDL cholesterol.

Conclusion: Elevated hs-CRP, MDA levels are potentially important diagnostic markers for the assessment of endothelial dysfunction in type 2 diabetic patients. Tight blood glucose control, regular monitoring of hs-CRP, MDA levels within normal range might be useful for reduction of vascular complications in type 2 diabetic patients.

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1. Introduction

Type 2 diabetes mellitus is a major public health problem worldwide and accompanied by enduring vascular complications, which leads to morbidity and mortality. Inflammation plays a pivotal role in the development of type 2 diabetes and vascular complications. Impaired insulin secretion and sensitivity leads to oxidative stress, endoplasmic reticulum stress, amyloid deposition in the pancreas, lipotoxicity and glucotoxicity. Studies reported that insulin resistance, inflammatory biomarkers, metabolic syndrome, dyslipidaemia, hypertension are predictive markers of cardiovascular disease (CVD) in type 2 diabetes mellitus. Chronic hyperglycemia and oxidative stress increases the pro-inflammatory proteins with infiltrated macrophages secreting inflammatory cytokines which leads to systemic inflammation. Hs-C-reactive protein is an acute phase reactant protein produced by liver response to several cytokines and sensitive marker of low grade systemic inflammation. Studies reported that hs-CR directly binds to oxidized low-density lipoprotein cholesterol (LDL-C), induces plasminogen activator inhibitor-1 expression,
endothelial dysfunction by which leads to cardiovascular disease (CVD). 9–11

Hyperglycemia induced oxidative stress induces pro inflammatory reagents with infiltrated macrophages secreting inflammatory cytokines which leads to local and systemic inflammation.12 It has been recognized high levels of free radicals or reactive oxygen species (ROS), reactive nitrogen species (RNS) directly damage to the lipids which leads to formation of aldehydes such as malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE).13,14 So, in this view the objective of present study was to evaluate hs-CRP, MDA levels in type 2 diabetic patients and also to explore their association with HbA1c and insulin resistance.

2. Materials and Methods

Fifty type 2 diabetic patients of both sexes aged between 35-45 years on oral hypoglycemic drugs, attending Department of General Medicine, Nimra Institute of Medical sciences, Jupudi, Andhra pradesh state, India were selected for present study. We excluded the patients on insulin, smokers, alcoholics, tobacco chewers, renal disease, inflammatory disorders, neoplastic disorders, thyroid disorders, liver disorders, history of acute myocardial infarction, stroke, and occlusive peripheral vascular disease. Fifty healthy age and sex matched subjects were selected as controls. The informed consent was obtained from all the study subjects and the study was approved by the Institutional Human Ethics Committee (IHEC). Experiments were done in accordance with Helsinki declaration of 1975.

2.1. Biochemical analysis

Fasting venous blood samples were collected from the study subjects and centrifuged at 3000 rpm for 15 min. Routine laboratory investigations were carried out by standardized protocols with ERBA EM-360 fully automated analyzer. Serum insulin estimated by Enzyme Linke d Immuno Sorbe nt Assay (ELISA), HbA1c estimated by (Ion Exchange Resin method) hs-CRP was assessed by (latex turbidimetric immunoassay) , malondialdehyde (MDA) estimated by Thiobarbituric Acid Reactive Substances (TBARS) method.15 Post prandial venous blood sample collected for plasma glucose (PPG) analysis.

Homeostasis model assessment for Insulin Resistance (HOMA-IR) HOMA-IR calculated by using fasting glucose and insulin values: HOMA – IR= fasting insulin X fasting glucose (m M/L)/22.516

2.2. Statistical analysis

Statistical analysis carried out with SPSS 25.0 software and values were expressed as mean ± standard deviation, p value < 0.05 was considered as statistical significant. Pearson correlation test performed for correlation analysis.

3. Results

| Parameters | Controls (n=50) | T2DM (n=50) | p value |
|------------|----------------|-------------|---------|
| Age (yrs)  | 39.5±4.9       | 38.3±6.7    | 0.48    |
| Body mass index (BMI) | 24.5±1.7 | 27.1±2.8 | 0.01    |
| Waist/Hip ratio | 0.90±0.05 | 0.93±0.08 | 0.02    |
| Systolic BP (mmHg) | 115.7±6.9 | 118.5±10.5 | 0.09    |
| Diastolic BP (mm Hg) | 78.1±5.4 | 80±8.2 | 0.15    |

Data are expressed as mean ±SD, p value <0.05 was considered statistically significant.

| Parameters | Controls (n=50) | T2DM (n=50) | p-value |
|------------|----------------|-------------|---------|
| FPG (mg/dl) | 81.6±8.9 | 135.0±12.6 | 0.001   |
| PPG (mg/dl) | 105.8±9.6 | 190±21.7 | 0.001   |
| HbA1C | 5.2±0.4 | 8.9±0.7 | 0.001   |
| HOMA-IR | 1.4±0.3 | 4.2±0.8 | 0.001   |
| Serum cholesterol (mg/dl) | 178.9±9.7 | 206.3±21.4 | 0.001 |
| Serum Triglycerides (mg/dl) | 98.9±10.3 | 134.1±16.7 | 0.001 |
| HDL cholesterol (mg/dl) | 43.0±2.4 | 40.2±4.8 | 0.02    |
| LDL cholesterol (mg/dl) | 108.10.5 | 134.0±15.3 | 0.001 |
| Total Bilirubin (mg/dl) | 0.07±0.09 | 0.79±0.05 | 0.75    |
| Direct Bilirubin (mg/dl) | 0.2±0.07 | 0.19±0.08 | 0.45    |
| AST (IU/L) | 28.6±3.5 | 28.8±5.4 | 0.67    |
| ALT (IU/L) | 28.4±3.9 | 30.5±5.7 | 0.08    |
| ALP (IU/L) | 98.6±12.1 | 99.4±14.7 | 0.17    |
| Serum urea (mg/dl) | 23.5±4.3 | 27.8±7.8 | 0.23    |
| Serum creatinine (mg/dl) | 0.68±0.4 | 0.77±0.5 | 0.322   |
| Hs-CRP (mg/L) | 1.9±0.4 | 4.8±1.8 | 0.001   |
| MDA (µ mol/L) | 1.9±0.6 | 6.5±1.4 | 0.001   |

Data are expressed as mean ±SD, p value <0.05 was considered statistically significant.

4. Discussion

Oxidative stress stimulates the inflammatory mediators which in turn enhances the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Oxidative stress induces tumour necrosis factor alpha (TNF-α) secretion, it is linked to obesity related insulin
and as well as decreased high-density lipoprotein (HDL) patients as reported earlier studies. High triglyceride levels eventually precedes type 2 diabetes mellitus.22 Results in metabolic derangements, insulin resistance and vascular complications in type 2 diabetes mellitus.23–25 The present study has been shown significant increased hs-CRP and MDA levels in T2DM patients compared with healthy controls. Body mass index and (BMI) and Waist hip ratio we observed significantly increased MDA levels in T2DM patients compared to healthy controls and also positive correlation with HbA1c and HOMA-IR. HbA1c is widely used as mean glycemic index in diabetes and also useful measurement for the vascular complications. Oxidative stress plays a crucial role in pathogenesis of diabetic vascular complications. Chronic hyperglycemia in diabetic patients can increase production of free radicals through Amadori rearrangement. In general, the ROS and RNS are continuously generated in physiological conditions and are eliminated by several antioxidant enzymes. Co-existence of inflammation, increased oxidative stress and vascular complications in type 2 diabetes mellitus.17,18 Several studies explored that oxidative stress is not only due to free radical generation and also due to nonenzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism, decreased antioxidant capacity.19–21 The present study was shown significant increased hs-CRP and MDA levels in T2DM patients compared with healthy controls.

Table 3: Correlation between hs-CRP & measured parameters in type 2 diabetic patients

| Parameters | Correlation Coefficient(r) |
|------------|-----------------------------|
| BMI        | 0.625**                     |
| W/H ratio  | 0.213                       |
| FBS        | 0.321*                      |
| PPBS       | 0.203                       |
| HbA1C      | 0.515**                     |
| HOMA-IR    | 0.493**                     |
| Cholesterol| 0.262                       |
| TGL        | 0.313*                      |
| HDL        | -0.356*                     |
| LDL        | 0.178                       |
| MDA        | 0.645**                     |

**Correlation is significant at the 0.01 level (2-tailed).  
*Correlation is significant at the 0.05 level (2-tailed).

Table 4: Correlation between MDA & measured parameters in type 2 diabetic patients

| Parameters | Correlation Coefficient(r) |
|------------|-----------------------------|
| BMI        | 0.398**                     |
| W/H ratio  | 0.293*                      |
| FBS        | 0.613**                     |
| PPBS       | 0.198                       |
| HbA1C      | 0.421**                     |
| HOMA-IR    | 0.539**                     |
| Cholesterol| 0.208                       |
| TGL        | 0.313*                      |
| HDL        | -0.294*                     |
| LDL        | 0.126                       |

**Correlation is significant at the 0.01 level (2-tailed).  
*Correlation is significant at the 0.05 level (2-tailed).

ROS and RNS are collectively used to describe free radicals and other non-radical reactive derivatives known as oxidants. Biologically free radicals are highly unstable molecules which are products of normal cellular metabolism. Oxidative stress induced DNA damage markers such as 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-oxo-7, 8-dihydro-2'-deoxyguanosine; lipid-peroxidation products measured as thiobarbituric acid reactive substances (TBARS). In the present study we observed significantly increased MDA levels in T2DM patients compared to healthy controls and also positive correlation with HbA1c and HOMA-IR. HbA1c is widely used as mean glycemic index in diabetes and also useful measurement for the vascular complications. Oxidative stress plays a crucial role in pathogenesis of diabetic vascular complications. Chronic hyperglycemia in diabetic patients can increase production of free radicals through Amadori rearrangement. In general, the ROS and RNS are continuously generated in physiological conditions and are eliminated by several antioxidant enzymes. Co-existence of inflammation, increased lipid peroxidation, dyslipidemia along with hyperglycemia conditions could pathologically increase the effect of oxidative stress. However, the decreased efficiency of cellular antioxidant mechanisms with simultaneously enhanced lipid peroxidation along with increased insulin resistance and HbA1c may contribute factors of provoking inflammatory pathways and vascular complications in type 2 diabetes mellitus.
5. Conclusion
Elevated hs-CRP, MDA levels are potentially important diagnostic markers for the assessment of endothelial dysfunction in type 2 diabetic patients. Tight blood glucose control, regular monitoring of hs-CRP, MDA levels within normal range might be useful for reduction of vascular complications in type 2 diabetic patients.

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None.

7. Conflict of interest
None.

References
1. Festa A, D’Agostino R, Howard G, Mykkänen L, Tracy RP, et al. Chronic Subclinical Inflammation as Part of the Insulin Resistance Syndrome. Circion. 2000;102(1):42–47. doi:10.1161/01.cir.102.1.42.
2. Donath MY, Shoeshorn SE. Type 2 diabetes as an inflammatory disease. Nature Rev Immunol. 2011;11(2):98–107. doi:10.1038/nri3002.
3. Oguntimeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol. 2019;11(3):45–63.
4. Antonopoulos AS, Tousoulis D. The molecular mechanisms of obesity paradox. Cardiovascular Research. 2017;113(9):1074–1086. Available from: https://doi.org/10.1093/cvr/cvx100.
5. Thorand B, Löwel H, Schneider A. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study. Arch Intern Med. 1984;163:93–102.
6. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005;115:1111–120.
7. Ford ES. The metabolic syndrome and C-reactive protein, fibrinogen, and leukocyte count: findings from the Third National Health and Nutrition Examination Survey. Atherosclerosis. 2003;168(2):351–559.
8. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001;286(3):327–334.
9. Devaraj S, Xu DY, Jialal I. C-Reactive Protein Increases Plasminogen Activator Inhibitor-1 Expression and Activity in Human Aortic Endothelial Cells. Circulation. 2003;107(3):398–404. Available from: https://dx.doi.org/10.1161/01.cir.0000082267.91920.0c.
10. Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, et al. Elevated C-reactive Protein Levels and Impaired Endothelial Vasoreactivity in Patients With Coronary Artery Disease. Circ. 2000;102(9):1000–1006. doi:10.1161/01.cir.102.9.1000.
11. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events. An 8-year follow-up of 14,719 initially healthy American women. ACC Curr J Rev. 2003;12(3):33–34. doi:10.1016/j.accu.2003.06.003.
12. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005;115:1111–1119.
13. Moldovan L, Moldovan NI. Oxygen free radicals and redox biology of organelles. Histochem Cell Biol. 2004;122(4):395–412. doi:10.1007/s00418-004-0676-y.
14. Cheseman KH, Beavis A, Estebauer H. Hydroxyl-radical-induced iron-catalysed degradation of 2-deoxyribose. Quantitative determination of malondialdehyde. Biochem J. 1988;252(3):649–653. doi:10.1042/bj0252649.
15. Mahlouz MOJ, Haniprasad CH, Shaffe IA, Sadasivudu B. Serum malondialdehyde levels in myocardial infarction and chronic renal failure. IRCS Med Sci. 1986;14:1110–1111.
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and ?-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–419. doi:10.1007/bf02680662.
17. Zhang P, Zhang X, Brown J, Vistisen D, Siree R, et al. Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87(3):293–301. doi:10.1016/j.diabetres.2010.01.026.
18. Derosa G, D’Angelo A, Bonaventura A, Bianchi L, Romano D, et al. Effects of berberine on lipid profile in subjects with low cardiovascular risk. Expert Opin Biol Ther. 2013;13(4):475–482. doi:10.1517/14712598.2013.776037.
19. Rosen P, Nawroth PP, King G, Miller W, Triggscher HJ, et al. The role of oxidative stress in the onset and progression of diabetes and its complications: summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. Diabetes/Metab Res Rev. 2001;17(3):189–212. doi:10.1038/sj.dmr.3700694.
20. Manna P, Jain SK. Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. Metabolic Syndrome and Related Disorders. 2015;13(10):423–444. Available from: https://dx.doi.org/10.1016/j.msdr.2015.09.003.
21. Lee DH, Ha MH, Kim HH. Gamma-glutamyltransferase and diabetes: a 4 year follow-up study. Diabetologia. 2003;12:359–364.
22. Gregor MF, Hotamisligil GS. Inflammatory Mechanisms in Obesity. Ann Rev Immunol. 2011;29(1):415–445. doi:10.1146/annurev-immunol-031210-102632.
23. Visscher TL, Seidell JC. The Public Health Impact of Obesity. Ann Rev Public Health. 2001;22(1):355–375. doi:10.1146/annurev.publhealth.22.1.355.
24. Ouwens DM, Sell H, Greulich S, Eckel J. The role of epicardial and perivascular adipose tissue in the pathophysiology of cardiovascular disease. J Cell Mol Med. 2010;14(9):2223–2234. doi:10.1111/j.1582-4934.2010.00889.x.
25. Zhang H, Cui J, Zhang C. Emerging role of adipokines as mediators in atherosclerosis. World J Cardiol. 2010;2(11):370–376.
26. Jisiecka-Onuigbo NN, Kalu OA, Onuigbo PC, Unuigbe EI, Ogudejo CO. Prevalence of dyslipidemia among adult diabetic patients with overt diabetic nephropathy in Anambra state South-East Nigeria. Nigerian J Clin Pract. 2011;14(2):171–175. doi:10.4103/1119-3580.81338.
27. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nature Reviews Endocrinology. 2009;5(3):150–159. Available from: https://dx.doi.org/10.1038/nrendo.2009.1.
28. Esteve E, Ricart W, Fernández-Real JM. Dyslipidemia and inflammation: an evolutionary conserved mechanism. Clin Nutr. 2005;24(1):16–31. doi:10.1016/j.clnu.2004.03.004.
29. Hirosami J, Tuncman G, Chang L, Gorgín CZ, Uysal KT, et al. A central role for INK in obesity and insulin resistance. Nature. 2002;420(6913):333–336. Available from: https://dx.doi.org/10.1038/35080572.
30. Nishimura S, Manabe I, Nagasaki M. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med. 2009;15:914–934.
31. Mehdiipur M, Zenouz AT, Davoodi F, Gholizadeh N, Damghani H, et al. Evaluation of the Relationship between Serum Lipid Profile and Oral Lichen Planus. J Dent Res, Dent Clin, Dent Prospects. 2015;9(4):261–266. doi:10.4103/1471-2598.15852.
32. Schaefer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radical Biol Med. 2001;30(11):1191–1212. doi:10.1016/s0891-5849(01)00306-x.
33. Giugliano D, Ceriello A, Paolizzo G. Oxidative Stress and Diabetic Vascular Complications. Diabetes Care. 1996;19(3):257–267. doi:10.2337/diacare.19.3.257.
34. Pendyala G, Thomas B, Joshi S. Evaluation of total antioxidant capacity of saliva in type 2 diabetic patients with and without periodontal disease: A case-control study. North Am J Med Sci.
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