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International Journal of Clinical Biochemistry and Research

Journal homepage: www.innovativepublication.com

Original Research Article

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

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ARTICLE INFO

Article history:
Received 28-10-2019
Accepted 23-11-2019
Available online 13-03-2020

Keywords:
Type 2 diabetes mellitus (T2DM)
High sensitivity C- reactive protein (hs-CRP)
Malondialdehyde (MDA)
Glycosylated hemoglobin (HbA1C)
Insulin resistance (IR)

ABSTRACT

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 play 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 compare 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. Ser um 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 increa sed in type 2 diabetic patients com pared with healthy controls. Hs-CRP and MDA levels w ere shown significant positive correlation with glyc osylated 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.

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 type2 diabetes and vascular complications.[1] Impaired insulin secretion and sensitivity leads to oxidative stress, endoplasmic reticulum stress, amyloid deposition in the pancreas, lipotoxicity and glucotoxicity.[2] Studies reported that insulin resistance, inflammatory biomarkers, metabolic syndrome, dyslipidaemia, hypertension are predictive markers of cardiovascular disease (CVD) in type 2 diabetes mellitus.[3–5] Chronic hy perglycemia and oxidative stress increases the pro-inflammatory proteins with infiltrated macrophages secreting inflammatory cytokines which leads to systemic inflammation.[6] 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.[7,8] Studies reported that hs - CRP directly binds to oxidized low-density lipoprotein cholesterol (LDL-C), induces plasminogen activator inhibitor-1 expression,

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https://doi.org/10.18231/j.ijcbr.2020.012

endothelial dysfunction by which leads to cardiovascular disease (CVD).[9–11]

Hyperglycemia induced o xidative stress induces pro inflammatory reactants 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 dysfunction, 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 ve nous 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)/22.5[16]

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

Table 1: Comparison of baseline parameters in controls, type 2 diabetic patients

Parameters	Controls (n=50)	T2DM (n=50)	p value
Age	37.5±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.

Table 2: Comparison of FPG, PPG, HbA1C, HOMA-IR, Lipid profile, Liver profile, Renal profile hs-CRP and MDA levels in control and type 2 diabetic subjects

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)	0.77±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±5.7	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	0.7 7±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

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

resistance 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 glutat hione metabolism, decreased antioxidant capacity.[19–21] 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 re significantly increased in T2DM patients compared with healthy controls and also hs - CRP , MDA showed significant positive correlation with BMI. Obesity is considered as low-grade systemic inflammation, which results in metabolic derangements, insulin resistance and eventually precedes type 2 diabetes mellitus.[22] Obesity enhances sympathetic drive, increase vasomotor tone and hypertension; they precedes to metabolic abnormalities such as dyslipidemia, insulin resistance, inflammation, endothelial dysfunction and organ injury.[23–25]

The present study also exhibits dyslipidemias in T2DM patients as reported earlier studies. High triglyceride levels and as well as decreased high-density lipoprotein (HDL) cholesterol, most likely underlying cause of increased free fatty acid flux, insulin resistance and vascular complications in type 2 diabetes mellitus.[26,27] We have observed significantly increased total cholesterol, triglycerides, LDL-C and decreased HDL-C in T2DM patients compared with healthy individuals and also hs -CRP, MDA levels were positively correlated triglycerides and negatively correlated with HDL cholesterol.

In the present study we observed hs - CRP levels showed significant positive correlation with MDA, HbA1c and HOMA-IR. Chronic inflammation is potentially unifying mechanistic cause, accompanied by activation of major inflammatory pathways such as Jun N-terminal kinases (JNK) and the transcription factor NF-kappaB along with decreased HDL-cholesterol, with impairment in reverse cholesterol transport mechanism and parallel changes in apolipoproteins, enzymes, decreased anti-oxidant capacity.[28–30] Decreased HDL-Cholesterol and p hospholipids could stimulate a ccumulation of VLDL, which binds bacterial products and other toxic substances, resulting in hypertriglyceridemia. Furthermore, it promotes lipid peroxidation by peroxynitrite formation by decreasing endogenous antioxidant defenses and enhances the formation of atherosclerotic lesions.[31]

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 us ed 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.[32] Chronic hyperglycemia in diabetic patients can increase production of free radicals through Amadori rearr angement.[33] In general, the ROS and RNS are continuously generated in physiological conditions and are eliminated by several antioxidant enzymes. C o-existence of inflammation, increased lipid peroxidation, dyslipidemia along with hyperglycemia conditions could pathologically increase the effect of oxidative stress.[34,35] 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.

6. Source of funding

None.

7. Conflict of interest

None.

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Cite this article: Santhi T, Mahendran KB. **High-sensitivity C-reactive protein, Malondialdehyde and their association with Glycated hemoglobin (HbA1c)in type 2 diabetes patients**. *Int J Clin Biochem Res* 2020;7(1):57-61.