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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Abstract

Background: Evaluation of High-sensitivity C-reactive protein, malondialdehyde(MDA)levels in type 2diabetic patients compare with healthy controls and correlate these levels with glycated hemoglobin (HbA1C) and insulin resistance.**Materials and Methods**: A prospective evaluation study was carried out in the Department of Biochemistry, Netaji Subhas Medical College and Hospital, Patna, Bihar India. The study population consisted of 200 subjects divided in to two groups viz., diabetic patients (type 2 diabetic subjects; n=100) and non- diabetic participants (n=100). 100 male and 100 female were include in this study. The age of the patients of both sex were 30-50 years. 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 vitros 350fully automated analyzer. **Results**: The mean serum hs-CRP and MDA levels were significantly highin type 2 diabetic patients compared with healthy patients. Hs-CRP and MDA levels we are shown significant positive correlation with glycocylated 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

Keywords: Abdominal malignancies, Incidence, Intestinal obstruction, Perforation.

Introduction

Diabetes mellitus (DM) considered as a widespread global disease. Conferring to recent reports, about 171 million persons in the world with DM in the year 2000 and this number expected to increase to 366 million through 2030. This disease is correlated with reducing life expectancy and significant other illnesses due to its relationship with microvascular complications (ischaemic heart disease, stroke and peripheral vascular disease), as a result led to lessen life quality[1]. Glycated hemoglobin (HbA1c) represents the blood glucose average level within the past 3 months. Therefore, HbA1c is a very important biochemical parameter that provide long term status of blood glucose levels and monitoring tool for measuring glycemic control in Type – 2 diabetic patients[2]. HbA1c in general, developed when the hemoglobin joined with glucose in the blood and become glycated[3]. According to many studies, HbA1c levels could be used as an independent risk factor for stroke and Cardiovascular disease (CVD) in both healthy and diabetics persons. It has been found that a (0.2%) decrease of HbA1c level can lower the risk of CVD development by 10%[4]. Furthermore, many studies have revealed, newborns moms with high HbA1c levels are more likely suffering from development of CVD in the future[5]. Chronic hyperglycemia and oxidative stress increases the pro-inflammatory proteins with infiltrated macrophages secreting inflammatory cytokines which leads tosystemic

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inflammation [6]. HsC reactive protein is an acute phase reactant protein produced by liver response toseveral 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-1expression endothelial dysfunction by which leads to cardiovascular disease (CVD)[9-11]. Hyperglycemia induced oxidative 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]. 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.

Materials and methods

A prospective evaluation study was carried out in the Department of Biochemistry, Netaji Subhas Medical College and Hospital ,Patna, Bihar India from November 2019 to April 2020, after taking the approval of the protocol review committee and institutional ethics committee.

Study sample and inclusion/exclusion criteria

The study population consisted of 200 subjects divided in to two groups viz., diabetic patients (type 2 diabetic subjects; n=100) and non- diabetic participants (n=100). 100 male and 100 female were include in this study. The age of the patients of both sex were 30-50 years.We excluded the patients on insulin,smokers, alcoholics, tobacco chewers, renal disease, inflammatory disorders, neoplastic disorders, thyroid disorders, liver dysfunction, and history of acute myocardial infarction, stroke and occlusive peripheral vascular disease.

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 vitros 350 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[14]. Post prandial venous blood samples 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)/22.51[15].

Statistical analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2010) and then exported to data editor page of SPSS version 19 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics included computation of percentages and means. Test applied for the analysis was student t-test. The confidence interval and level of significance were set at 95% and 5%.

Results

Table 1: Gender and age distribution of patients

Variables	N=200	Percentage %
Gender		
Male	100	50
Female	100	50
Age		
30-40 years	80	40
40-50 years	120	60

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

Parameters	Controls(N=100)	T2DM(N=100)	p-value
Age	38.3±3.6	39.6±6.9	0.44 (NS)
Body mass index (BMI) kg/m^2	25.1±1.3	28.1±2.6	0.012*
Waist/Hip ratio	0.92±0.02	0.94±0.11	0.024*
Systolic BP(mmHg	116.3±5.7	119.2±9.4	0.08 (NS)
Diastolic BP (mm Hg)	77.1±5 .8	80±7.8	0.13 (NS)

*indicates statistical significance (≤0.05)

Table 3: 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)	80.9± 8.7	134.0±13.5	0.021*
PPG(mg/dl)	106 .2±8.8	189±23 .6	0.032*
HbA1C	5.1±0.6	8.7±0.9	0.026*
Serum Triglycerides (mg/dl)	97.7±11.1	135.1±14.4	0.031*
Serumcholesterol (mg/dl)	179.8±9.8	207.2±22.8	0.042*
HOMA-IR	1.3±0.2	3.9±0.9	0.039*
HDLcholesterol (mg/dl	44.0±1.9	40.1±3.1	0.027*
LDLcholesterol (mg/dl)	109±11.1	135.0±14.2	0.014*
Total Bilirubin(mg/dl	0.76±0.06	0.78±0.07	0.72 (NS)
Direct Bilirubin(mg/dl)	0.2±0.07	0.19±0.08	0.41 (NS)
Serumurea(mg/dl)	22.9±4.6	27.2±6.9	0.24 (NS)
Serum creatinine(mg/dl)	0.67±0.3	0.78±0.7	0.311 (NS)
Hs-CRP(mg/L)	1.8±0.3	4.1±1.7	0.018*
MDA(µmol/L	1.8±0.6	5.9±1.6	0.029*

Table 4: Correlation between hs-CRP &measured parameters in type 2 diabetic patients

Parameters	Correlation Coefficient(r)
BMI	0.615**
W/H ratio	0.209
FBS	0.315*
PPBS	0.199
HbA1C	0.511**
HOMA-IR	0.482**
Cholesterol	0.249
TGL	0.301*
HDL	-0.333*
LDL	0.162
MDA	0.638**

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

Table 5: Correlation between MDA &measured parameters in type 2 diabetic patient

Parameters	Correlation Coefficient(r)
BMI	0.389**
W/Hratio	0.288*
FBS	0.609**
PPBS	0.191
HbA1C	0.415**
HOMA-IR	0.543**
Cholesterol	0.218
TGL	0.310*
HDL	-0.286*
LDL	0.121

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

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 resistance and vascular complications in type 2 diabetes mellitus[16,17]. 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[18 – 20]. 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[21]. Obesity enhances sympathetic drive, increase vasomotor tone and hypertension; they proceed to metabolic abnormalities such as dyslipidemia, insulin resistance, inflammation, endothelial dysfunction and organ injury[22-24].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[25,26]. 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 HDLcholesterol 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 JunN-terminal kinases 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[27-29]. Decreased HDL-Cholesterol and phospholipids could stimulate accumulation of VLDL, which binds bacterial products and other toxic substances, resulting in hypertriglyceridemia.

Furthe rmore, it promotes lipid peroxidation by peroxynitrite formation bydecreasing endogenous antioxidant defences and enhances the formation of atherosclerotic lesions[30].ROS and RNS are collectively used to describe free radicals and other non-radical reactive derivatives known as oxidants. Biologically free radicalsare 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 complication[31]. Chronic hyperglycemia in diabetic patients can increase production of free radicals through Amadori rearr angement[32]. 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[33]. 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.

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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