Adiponectin Level Association with MDA in the Patients with Type 2 Diabetes Mellitus

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http://dx.doi.org/10.13005/bpj/1963

(Received: 31 October 2019; accepted: 25 January 2020)

MDA level were established to be augmented in poor controlled T2DM and is positively correlated with progression of diabetes while adiponectin is negatively correlated with diabetes progression furthermore adiponectin high level is associated with low risk of cardio-metabolic complication and low risk of macro vascular complication in patient with T2DM. To find out the correlation between Adiponectin and MDA level in patient with T2DM (type 2 Diabetes Mellitus). 120 sample size, age above 30yrs, was taken in present study from sub urban population grouped as T2DM, Prediabetic and healthy non diabetic as control group Mean ±SD (n=120) MDA =3.94±1.02 in Diabetic group, MDA=1.93±0.31 in prediabetic group and control group MDA = 2.80±1.51, While adiponectin level Mean ±SD (n=120) in diabetic group =3.94±1.02, adiponectin in prediabetic group=9.72±0.31 and the control group adiponectin level =10.81±2.24 Our study suggest that MDA level are augmented with progression of Diabetes Mellitus (T2DM) especially poor controlled T2DM ,MDA blood level are high in T2DM and prediabetic group as compared to control group while low level of adiponectin was found in prediabetic patient group and T2DM group indicating risk of diabetic complication as compared to controlled group which have high level of adiponectin . Also the study confirmed Adiponectin is a reliable biochemical indicator and its correlation in diabetic patient, it reduces the risk of diabetes and diabetic complication and metabolic syndrome and insulin resistance.

Keywords: Adiponectin; Lipid Peroxidation; Malondialdehyde (MDA); Oxidative Stress; Pre diabetes; Type 2 Diabetes Mellitus.

Diabetes mellitus is growing health issue with endless multiple complication and may lead to early death. Poor life style, obesity, aging, stress and several factors are implicated in the prevalence of the disease. The disease Diabetes Mellitus is established as a constellation of a number of metabolic disorders, and is characterized by hyperglycemia related to defects in insulin secretion and insulin action or both. The chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of normal functioning of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels1,2. Diabetes-specific microvascular disease is a leading cause of blindness, renal failure, and nerve damage 3. The prevalence of diabetes is rising all
over the world due to population growth, aging, urbanisation, and the increase of obesity due to physical inactivity. In the year 2000, the global number of individuals with diabetes was estimated to be 171 million (2.8% of the world’s population), and this figure has been projected to increase in 2030 to 366 million (6.5%), 298 millions of whom will be living in developing countries [WHO, 1998]. According to International Diabetes Federation (IDF) data, the total number of people in India with diabetes are reported to be 50.8 millions in 2010 and would be 87.0 million by 2030.

Adiponectin

Fatty tissue (adipose tissue) discharge a cytokine called Adiponectin, and enormously secreted in white adipose tissue. Adiponectin triggers 5’AMP initiated protein kinase and countless other protein enzymes kinases. Additionally AMPK pathway can increase glucose uptake and use and free fatty acid (FFA) oxidation in various tissue like skeletal muscle cells, hepatocyte (liver) and fatty tissue (adipose tissue), thus controlling rate of metabolism and insulin sensitivity. Adiponectin can also impede the mutilation on blood vessel especially the vascular endothelial cells from inflammatory elements and thus shield the cardiovascular system. Adiponectin is linked with many diseases as the study suggests it is linked to obesity, insulin resistance, T2DM etc.

Adiponectin Receptors

Adiponectin receptor 1 and adiponectin receptor 2 are of two main categories nominated as AdipoR1, AdipoR2. AdipoR1and AdipoR2 precisely expressed in a diverse tissue, but their dispersal is different. In vitro investigational facts suggest that AdipoR1 appearance is essentially seen in vascular endothelial cells and blood macrophages, signifying that in vascular pathophysiology AdipoR1 may be tangled.

Oxidative Stress and Diabetes

Oxidative stress plays a pivotal role in cellular injury from hyperglycaemia. Oxidative stress is principally discrepancy between the rate of production of free radicals and the capacity of the body system to counter or detoxify their detrimental effects through neutralization by antioxidants. It can cause noxious effects through the assembly of peroxides and free radicals that mutilate all apparatuses of the cell, including proteins, lipids, and DNA. High glucose level can stimulate free radical production. Fragile shielding system of the human body becomes incapable to impede the enhanced ROS generation and as a result condition of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress. Oxidative stress acts as mediator of insulin resistance and its progression to glucose intolerance and installation of diabetes mellitus, subsequently favouring the appearance of atherosclerotic complications, and contributes to rise in many micro and macrovascular complications.

Lipid Peroxidation

Lipids are reported as one of the primary targets of ROS. Peroxidation of lipids in cell membrane produces extremely reactive molecules like aldehydes, including MDA, acrolein, 4-hydroxynonenal (HNE), 4-oxononenal (ONE), and isolevuglandins (IsoLGs). Lipid peroxidation is the oxidative degradation of lipids. It is phenomenon of stealing of electrons from the lipid molecules present in cell membranes, leading to cell damage. This process occurs and executed through free radical chain reaction mechanism. Malondialdehyde is a highly reactive compound and results from lipid peroxidation of polyunsaturated fatty acids. Increased MDA level in plasma, serum, and many others tissues has been reported in diabetic patients. Furthermore, various antioxidant assays are described in vitro and vivo. Similar work on oxidative stress in diabetes mellitus and MDA level in diabetes and its correlation with HbA1c and altered biomolecules due to oxidative stress in diabetes is highlighted. Moreover the complication of poorly controlled blood glucose concentration in diabetic patient include micro and macrovascular, neurological and end organ damages like kidney, peripheral digits. Complications such
as diabetic retinopathy, nephropathy with various molecular target and various novel drugs for newly discovered molecular targets are under scanner of researcher, neuropathy, gastropathy and dyslipidaemia are currently in limelight and therapy to control and manage such complications with new mode of drug delivery by nanoparticles or by gene delivery or by gene silencing along with identification of reliable biomarker for prognosis of disease.

**MATERIALS AND METHODS**

**Study Design**
Cross Sectional Case Control study

**Study Group**
Controls, pre diabetic and Diabetic patients.

**Sample Size Calculation**
This study is a hospital based study. 120 subjects was selected in this study. The prevalence of diabetes in the previous study

Scheme 1. Various action for adiponectin at Cellular Levels
conducted in Uttar Pradesh region was 11.6%.41. Using this, the sample size n at 95% confidence and the error of 5 % is
\[ n = \left( \frac{z}{2} \right)^2 \times p \times (1-p)/c^2 \]

Place of Work
The study was carried out in the Department of Biochemistry, Rama Medical College Hospital & Research centre, Kanpur.

Study Population
Patients were selected from both outpatient and inpatient department in random method. The information collected is according to prescribed format.

Case definition of Diabetes Mellitus

Inclusion Criteria
1. Patients with type 2 Diabetes Mellitus (according to ADA criteria for diabetes)
2. Pre-diabetes (Impaired Fasting Glucose/Impaired Postprandial Glucose)
3. Age group > 30

Exclusion Criteria
1. Patients with any systemic disease e.g. asthma, chronic obstructive pulmonary disease (COPD), malignancies, Sexually Transmitted Diseases, cardiovascular disease
2. Patients with Type 1 diabetes mellitus.

Groups
Group I: Type 2 Diabetes Mellitus
Group II: Prediabetic Group
Group III: Healthy non diabetic group was used as control

METHODS

Collection of Sample
5 ml of venous (antecubital vein) blood collected after overnight fasting with all aseptic precautions in three parts. The first part was collected in Ethylene Diamine Tetra-Acetate (EDTA) vial for estimation of HbA1c. The second part was collected in plain vial for determination of serum Adiponectin and serum MDA.

Methodology
MDA and Adiponectin were estimated by kit based double-antibody sandwich enzyme-linked immunosorbent ELISA methods. Serum glucose was estimated by kit based glucose oxidase peroxidase method and HbA1c by nephelometry method.

Statistical Analysis
Appropriate statistical method was applied within the groups.

The results are presented in mean±SD and percentage. Percentage of test was used to compare

<table>
<thead>
<tr>
<th>Table 1. Frequency distribution among male and female in Diabetes and Prediabetes and Control subjects</th>
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<tbody>
<tr>
<td><strong>Diabetes Patients (n=120)</strong></td>
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<td>Female</td>
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<td>Male</td>
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<td>Total</td>
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<th>Table 2. Clinical Characteristics of Diabetes and Prediabetes and Control Subjects Participating in the Study</th>
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<td><strong>Diabetes Mean ±SD (n=120)</strong></td>
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<td>Age</td>
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<tr>
<td>MDA</td>
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<td>Adip.</td>
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the categorical variables between Diabetes, Prediabetes and Control. Unpaired t-test was used to compare the study parameters between cases and controls. The Pearson correlation coefficient was calculated among the study parameters. The p-value<0.05 was considered significant. All the analysis was carried out by using SPSS 21.0 version (Chicago, Inc., USA).

RESULTS

Table 1: In Diabetes Patients group, 38 (31.66%) patients were females and 82 (68.33%) were males whereas in Prediabetes Persons group, there were 29 (24.16%) males and 91 (75.83%) females. In Control subjects there were 52 (43.33%) males and 68 (56.66%) were females.

Table 2: In normal individual group, all the serum values were in normal range. In the clinical characteristics of Diabetes is higher in age group compared to Prediabetes (54.68±10.67 vs 47.23±2.06) Prediabetes to Control Subjects is below age group (47.23±2.06 vs 48.07±10.02). Diabetes is higher in MDA compared to Prediabetes (3.94±1.02 vs 2.80±1.51) Prediabetes to Control Subjects is below age group (2.80±1.51 vs 1.93±0.31). Diabetes is higher in MDA compared to Prediabetes (6.55±0.66 vs 9.72±0.31) Prediabetes to Control Subjects is below age group (9.72±0.31 vs 10.81±2.24).

Table 4 & Fig. 1-3: Shows the correlation matrix which represents the quantitative measurements of degree of relationship among different variables. The table showed that MDA and ADPN was negatively correlated with mild correlation (r=-.113, p=0.219) in cases. There is negative correlation between ADPN and MDA (r=-0.011 p=0.903). In figure association between MDA and Adiponectin in Diabetes Cases strongly correlated (r value = 0.013). Association between Adiponectin in Diabetes & Control Subjects are strongly correlated (r value = 0.016) MDA and Adiponectin in Diabetes & Control Subjects strongly correlated (r value = 0.003).

Table 4 & Fig. 4-6: Shows the correlation matrix which represents the quantitative measurements of degree of relationship among different variables. The table showed that diabetic and prediabetes was ADPN and ADPN was negative correlation mildly correlated (r=-.128, p=0.162) in cases. There is negative correlation between ADPN and MDA (r=-0.011 p=0.903) & MDA & ADPN was (r=0.057 p=0.538). Association between MDA and Adiponectin in Diabetes Cases strongly correlated (r value = 0.013). Association of prediabetes MDA and ADPN in Prediabetes Cases negative strongly correlation & strongly significant (r =-0.626** p=0.000). In figure association between ADPN and MDA in Prediabetes Cases correlated (r value = 1.266) Association between Adiponectin and Adiponectin in Prediabetes Cases & Control Subjects strongly correlated & significant (r=0.005). MDA and Adiponectin in Prediabetes Cases & Control Subjects (r=0.059)

Table 3. Pearson Correlation Coefficient among the Biochemical Parameters in Diabetes Cases

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<tr>
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<th>ADPN ng/ml</th>
<th>MDA ng/ml</th>
<th>ADPN ng/ml</th>
<th>MDA ng/ml</th>
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<tr>
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**. Correlation is significant at the 0.01 level (2-tailed). ADPN- Adiponectin, MDA- Malondialdehyde
Fig. 1. Scatter Diagram Showing Association between MDA and Adiponectin in Diabetes Cases

Fig. 2. Scatter Diagram Showing Association between Adiponectin and Adiponectin in Diabetes Cases & Control Subjects
Table 4 & Fig. 1-4: Shows the correlation matrix which represents the quantitative measurements of degree of relationship among different variables. The table showed that Diabetes cases & Control Subjects was MDA and ADPN was negative correlation mildly correlated \((r=-.011, \ p=0.903)\) in cases. There is negative correlation between ADPN and MDA and strongly significance \((r=-0.249 \ p=0.008)\). Prediabetes MDA and ADPN in diabetes & control group negative strongly correlation & strongly significant \((r =-0.626** \ p=0.000)\).

**. Correlation is significant at the 0.01 level (2-tailed). ADPN- Adiponectin, MDA- Malondialdehyde

**Table 4. Pearson Correlation Coefficient among the Biochemical Parameters in Diabetes & Prediabetes Cases**

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<thead>
<tr>
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<th>ADPN ng/ml</th>
<th>MDA ng/ml</th>
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<td>Sig. (2-tailed)</td>
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<td>ADPN ng/ml</td>
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<td>Pearson Correlation</td>
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Fig. 3. Scatter Diagram Showing Association between MDA and Adiponectin in Diabetes Cases & Control Subjects
**Fig. 4.** Scatter Diagram Showing Association between MDA and Adiponectin in Prediabetes Cases

**Fig. 5.** Scatter Diagram Showing Association between Adiponectin and Adiponectin in Prediabetes Cases & Control Subjects
**DISCUSSION**

Adiponectin gene is traced in human being on chromosome 3q27 region, which has been establish to be linked with T2DM and metabolic syndrome. It has a molecular weight of 30kDa and the programming gene encompasses 244 amino acids four areas of Adiponectin cytokine encompasses of the N-terminal signal peptide area, the non-helical functional area, the collagen area and the carboxyl terminal globular area. Foregoing research have presented that serum adiponectin level were considerably declined in persons with metabolic syndrome, coronary heart disease obesity, T2DM, and hypertension.

**Table 5.** Pearson Correlation Coefficient among the Biochemical Parameters in Diabetes cases & Control Subjects

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<td>.423</td>
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<tr>
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<td>.244**</td>
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**. Correlation is significant at the 0.01 level (2-tailed). ADPN- Adiponectin, MDA- Malondialdehyde**

**Fig. 6.** Scatter Diagram Showing Association between MDA and Adiponectin in Prediabetes Cases & Control Subjects
In present research study, serum Adiponectin level prediabetic groups and in T2DM were established with low level than that in controlled group. Related with healthy controls, the T2DM cases revealed substantially lower level of serum Adiponectin, which was reliable and verified with results from preceding exploration. Yamauchi T et al establish that Adiponectin might improve muscle FFA oxidation and energy utilization, and decrease the level of TG in Hepatocytes and skeletal muscle cells, thus retreating insulin resistance.

Maeda N et al. [50] testified that Adiponectin could instigate various cells especially skeletal muscles, hepatocytes to insulin via increasing muscle free fatty acid oxidation and hindering glucose output from liver as demonstrated in animal study by genetic manipulation especially knocking out Adiponectin gene in mice. More over adiponectin can govern carbohydrate metabolism and increase insulin sensitivity in peripheral tissues. In our investigation, serum Adiponectin concentration in blood displayed highly positive correlation between the diabetic and control group and prediabetic and control group P < 0.01, which was reliable with the preceding exploratory studies.

Furthermore, our study displayed T2DM patients and prediabetic patient have low blood level of adiponectin as compared to healthy individual similarly serum MDA blood level were high in T2DM and prediabetic patient as compared to normal healthy individuals thus suggesting both MDA and blood level of adiponectin are reliable forecaster biochemical parameter to analyse the progression of T2DM. Nilsson PM et al. also got equivalent deduction, and stated that the blood level of Adiponectin was negatively linked with carotid artery intima-media width, signifying that Adiponectin was a biochemical parameter and reliable indicator for subclinical cases of atherosclerosis and might forecast the risk for myocardial infarction and cerebral stroke.

Jiang et al. testified that in patient with peripheral vascular disease with onset in T2DM the blood level of adiponectin was negatively associated with disease. Though, there were still some deficiencies in present research. Primarily, the sample size is taken from specific geographical location and other confounding factor which might alter the blood level of MDA and adiponectin may factor which might alter the reading in specified subset of population. Furthermore, the molecular and cellular mechanism of Adiponectin and its receptor signalling pathway in regulating carbohydrate metabolism, lipid metabolism, immunological effect, anti-proliferative effect, anti-thrombotic action and T2DM is still area to explore in detail.

CONCLUSION

Presently, adiponectin is amongst the durable and best reliable as a dual biochemical marker forecaster of type 2 DM and Obesity patients. Adiponectin low level are associated with high risk for cardio-metabolic complication and macro vascular complication in patient with T2DM. MDA level where established to be augmented in poor controlled T2DM and is positively correlated with progression of diabetes while.

We concluded this study was higher levels of circulating adiponectin are associated with a lower risk of type 2 diabetes, poor control of type 2 diabetes. For clinical investigation in adiponectin parameter reveal its potential as a biomarker in assessment of prediabetes state and T2DM screening.

ACKNOWLEDGEMENT

All authors are very helpful to the department of Biochemistry, Rama Medical College, Hospital & Research Centre Kanpur, India for help in writing the paper.

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