Significance Of Interleukin-6 In Diabetes Mellitus And Its Complications

A. Mary Chandrika1, Dr. V.S.Kalai Selvi2

1Assistant Professor, Department of Biochemistry, Sree Balaji Medical College and Hospital, Chrompet, Chennai-600044.
2Professor, Department of Biochemistry, Sree Balaji Medical College and Hospital, Chrompet, Chennai-600044

Abstract: Low grade inflammation is one of the causes in the pathogenesis of type 2 diabetes. Interleukin – 6 (IL-6) a pro-inflammatory cytokine is found to be increased in type 2 diabetic patients. This study was aimed to assess the role of Interleukin-6 in type 2 diabetes mellitus. This was a cross sectional study done among diabetics and non-diabetics. The total number of subjects involved in the study was 120 within the age group of 20-50 years attending the outpatient department and diabetic clinic of Sree Balaji Medical College and Hospital. The study group comprised of Group- I – 40, age and sex matched non diabetic subjects. Group- II – 40, type II diabetic patients with good control of blood glucose, Group III – 40, type II diabetic patients with poor glycemic control. Blood samples were taken after 8 hours of fasting period, centrifuged at 3000 rpm for 10 min and serum, plasma separated. Plasma glucose levels (FBG, PPBG) were estimated by GOD/POD method, IL- 6 levels were measured by ELISA method and HbA1c by immunoturbidimetric method. Data was statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 18. Results showed that the circulating Interleukin- 6 (IL-6) levels were found to be elevated in type 2 diabetics than non diabetic subjects (p < 0.0001). Raised level of Interleukin-6 was found in diabetics compared to non diabetics that showed the presence of inflammatory mechanisms in diabetes patients. Also uncontrolled diabetics have increased Interleukin-6 levels. In addition to periodic checking of HbA1c, it is paramount important to check the inflammatory status in high risk subjects for diabetes and also in diabetics to prevent diabetic complications.

Keywords: Interleukin- 6; glycated hemoglobin, type 2 diabetes mellitus; HbA1C, diabetic complications.
1. INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia caused by a defect in insulin secretion, insulin action, or both. Several pathophysiology theories have been proposed to explain the onset of diabetes mellitus. Several studies have found that low-grade inflammation is a risk factor for the development of type 2 diabetes mellitus in the future. The precise mechanism is not known. Diabetes type 2 is linked to aging, obesity, and inactivity. It is caused by the progressive failure of beta cells in the pancreatic islets to compensate for insulin resistance. Various mechanisms, including oxidative stress, ectopic lipid deposition in muscles, liver, and pancreas, lipotoxicity, and glucotoxicity, have been proposed to explain impaired insulin resistance and sensitivity in type 2 diabetes. All of these stresses have the potential to cause an inflammatory response. Other mechanisms include hypoxia, nuclear factor activation, and interleukin activation. Immunological research examines the potential role of inflammation in the pathogenesis of type 2 diabetes. Interleukin-6 (IL-6), a pro-inflammatory cytokine, is responsible for the pathology of type 2 diabetes mellitus through insulin resistance by controlling differentiation, migration, proliferation, and cell apoptosis. IL-6, apart from its immuno-regulatory actions, has been suggested to affect glucose homeostasis and metabolism directly and indirectly by the action on skeletal muscle, adipocytes, hepatocytes, pancreatic beta cells and neuro-endocrine cells. Immuno-regulatory mediators not only cause metabolic aberrances but also cause beta cell dysfunction and insulin resistance. IL-6 associated with obesity is due to accumulation of macrophages in adipose tissue which release inflammatory mediators promoting inflammation. A low-grade inflammation is one of the causes in the pathogenesis of type 2 diabetes mellitus. IL-6 is a multifunctional pro-inflammatory cytokine produced by adipocytes, endothelial cells, smooth muscle cells, fibroblasts, lymphocytes and macrophages. It affects insulin sensitivity and regulation, lipoprotein lipase action, adipocyte function and thus decreases islet cell secretory function. Certain studies emphasize that endothelium is the target tissue of insulin and therefore Insulin Resistance also exists at the level of endothelium. Insulin promotes vasodilation by activating the signaling pathway involving IR, IRS 1 and PI3 kinase which is similar to that stimulate insulin mediated glucose uptake in tissues. Few studies have addressed the effect of IL6 on Insulin signaling pathway causing impairment of insulin signal transduction in hepatocytes and adipocytes. The potential role of IL6 in vascular insulin is still not yet clear. Previous studies highlight the role of Interleukin 6 as a pleiotropic cytokine involved in inflammation. Diabetes Mellitus is one of the major endocrine disorders for which low-grade inflammation forms the pathogenesis. This study was planned to confirm the same finding in our population which might pave the way for development of Anti IL6 monoclonal antibodies as a treatment modality in type 2 Diabetes Mellitus. This study was aimed to evaluate the role of Interleukin 6 in type 2 diabetes mellitus and its complications. The objectives of the study were to evaluate the association of IL-6 in type 2 diabetes mellitus and to evaluate the significance of IL-6 in patients uncontrolled diabetes mellitus.

2. MATERIALS AND METHODS

This was a cross-sectional study done among diabetics and non-diabetics. The total numbers of subjects involved in the study were 120 within the age group of 20-50 years attending the outpatient department and diabetic clinic of Sree Balaji Medical College and Hospital. The study group comprised of Group- I - 40 age and sex matched non diabetic subjects. Group- II -40 type II diabetic patients with good control of blood glucose, Group III -40 type II diabetic patients with poor glycemic control. All the study participants were given a proforma to collect the detailed history. Clearance obtained from the Institutional Ethical Committee (REF NO.002/SBMC/IHEC/2017/930) and informed consent obtained from all those who have been involved in the study. Under aseptic precaution blood samples were collected for analysis:

2.1 Inclusion criteria

- Type 2 diabetes mellitus patients and Non diabetic subjects of 20-50 years of age.
- Willing to participate in the study.

2.2 Exclusion criteria

- Patients with acute infection, chronic inflammatory diseases, obesity, cancer, elevated liver indices
- Patients with high serum creatinine levels of >1.5 mg/dl, pregnancy, arthritis and those on corticosteroids.

2.3 Biochemical analysis

Blood samples were taken after 8 hours of fasting period, centrifuged at 3000 rpm for 10 min and serum, plasma separated. Plasma glucose levels (FBG, PPBG) were estimated by GOD/POD method, IL-6 levels were measured by ELISA method and HbA1c by Immunoturbidimetric method.

3. STATISTICAL ANALYSIS

The characteristics of study participants are summarized using mean and standard deviation. The outcome variables namely, fasting plasma glucose, postprandial plasma glucose, glycated hemoglobin (HbA1c), serum IL6 were compared among three groups by Bonferroni method. A two sided p-value less than 0.05 was considered to be statistically significant. All the results data were statistically analyzed using SPSS version 18.

4. RESULTS

Forty Non diabetic persons were included in Group I with mean age 30.4 years and SD 7.4. Forty Type 2 Diabetic patients with good Glycemic control as Group II with mean age 32.8 years and SD 7.9. Forty Type II Diabetic patients without Glycemic control as Group III with mean age 35.4 years and SD 9.6 were included. In Group I the mean Fasting Blood Glucose 95.3 mg/dl with SD 8.5 and Postprandial Blood Sugar 103 mg/dl with SD 7.4 In Group II the mean Fasting Blood Glucose 114.2 mg/dl with SD 6.9 and Postprandial Blood Sugar 118.6 mg/dl with SD 8.6 In Group III the mean Fasting Blood Glucose 137.2 mg/dl with SD 17.0 and Postprandial Blood Sugar 190.7 mg/dl with SD 44.5. Mean HbA1C in Group I -5.5% with SD 0.3 Mean HbA1C in Group II -6.3% with SD 0.4. Mean HbA1C in Group III -7.2% with SD 0.7. Mean Interleukin 6 in Group I 4.6 pg/ml with SD 0.3. Mean Interleukin 6 in Group II 7.3 pg/ml with SD 1.3.
Mean Interleukin 6 in Group III 12.8pg/ml with SD 2.0. All the results were tabulated in Table I.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
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<th>Group III</th>
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<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>7.4</td>
<td>32.8</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>95.3</td>
<td>8.5</td>
<td>114.2</td>
</tr>
<tr>
<td>PPG (mg/dl)</td>
<td>103</td>
<td>7.4</td>
<td>118.6</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>5.5</td>
<td>0.3</td>
<td>6.3</td>
</tr>
<tr>
<td>IL 6 (pg/ml)</td>
<td>4.6</td>
<td>0.3</td>
<td>7.3</td>
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SD- standard deviation; FPG- fasting plasma glucose; PPG- postprandial plasma glucose; HbA1C – glycated hemoglobin; IL 6 – interleukin 6.

Group I – Non diabetic subjects
Group II - type II diabetic patients with good control of blood glucose
Group III – type II diabetic patients without blood glucose control.

The mean age of participants was 33± 8.3 years. The mean FPG, PPG, HbA1c and IL 6 levels were higher in group III compared to the other groups.

Comparison of Glycated hemoglobin between the groups I and II show a p value of less than 0.0001.
Comparison of Glycated hemoglobin between the groups I and III show a p value of less than 0.0001.
Comparison of Glycated hemoglobin between the groups II and III show a p value of less than 0.0001.

<table>
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<tr>
<th>GROUP</th>
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<tr>
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<tr>
<td>III</td>
<td>&lt;0.0001</td>
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</table>

Data in the table are p values

Comparison of Interleukin 6 between the groups I and II show a p value of less than 0.0001.
Comparison of Interleukin 6 between the groups I and III show a p value of less than 0.0001.
Comparison of Interleukin 6 between the groups II and III show a p value of less than 0.0001.

The mean of interleukin 6 level was significantly different between groups I and II, groups II and III, groups I and III (all p values <0.05) as in table III.

<table>
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<tr>
<th>GROUP</th>
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<tr>
<td>II</td>
<td>&lt;0.0001</td>
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<tr>
<td>III</td>
<td>&lt;0.0001</td>
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</tbody>
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Data in the table are p values

Comparison of HbA1c and fasting blood glucose of our study is consistent with the study by He Q et. Al.10
The findings of a meta analysis by Nicholas Bowker et. al., is consistent with the findings of this study11. Studies by Pradhan et al. have demonstrated an increase of CRP and IL-6 in individuals with features of insulin resistance and clinically overt type 2 diabetes mellitus. The study also revealed that CRP is a more powerful inflammatory marker than IL-6. In this CRP was not done.

5. DISCUSSION

The pathogenesis of type 2 Diabetes is characterized by a combination of Insulin Resistance and failure of pancreatic beta cells to compensate for the increased insulin demand. During the past decades, much evidence supports the concept that the insulin resistance and type 2 Diabetes Mellitus are related to chronic low grade inflammation. Circulating IL 6 levels have been reported to be elevated in patients with type 2 Diabetes and correlate with the direct and indirect measures of Insulin Resistance. IL-6 is one of the major inflammatory cytokines produced by a variety of tissues like activated leukocytes, adipocytes & endothelial cells. IL 6 is one of the mediators of acute phase response, and also enhances the release of hepatic C- reactive protein in the liver. The result obtained from this study shows that HbA1c in uncontrolled diabetic patients was significantly correlated with IL-6 but this observation was not seen in
non-diabetic subjects. Persistent hyperglycemia contributes to the formation of advanced glycation end products, up-regulation of the innate immune system resulting in chronic inflammation. This association among glycated hemoglobin (HbA1c), IL-6 suggests the role of a prominent inflammatory mechanism in type 2 diabetes mellitus. This study shows that IL 6 is elevated in diabetic patients than the diabetic subjects. Zahran et al and Spranger J et al have also mentioned similar results in their studies. Elevated levels of IL-6 were observed in both poorly and well-controlled diabetes in many independent clinical studies. Pradhan et al 15, in their study stated that type 2 diabetes mellitus may be a manifestation of an ongoing cytokine-mediated acute response initiated by the body’s innate immune system. Nalysnyk et al, indicated that not only poor glycemic control but also enhanced production of IL-6 leads to microvascular complications. IL-6 participates in pathogenesis of endothelial dysfunction by stimulation of monocyte chemotactic protein-1 and cell adhesion molecules. Some clinical results strongly suggest that enhanced IL-6 concentration is associated with diabetic kidney disease-enhanced stimulation of mesangial cell proliferation which interferes with the extracellular dynamics of matrix formation at the podocyte level. Christian Herder et al, in their study had revealed associations between biomarkers of subclinical inflammation and depressive symptoms in patients with diabetes mellitus. Meta analytic studies performed, provide a better understanding that IL-6 produced by a variety of cells involved in inflammation and act as signals in pathological processes associated with chronic inflammation. Studies reveal circulatory levels of IL-6 and TNF- are elevated in diabetic patients. Hyperglycemia increases circulating cytokine concentration by oxidative mechanism. Also numerous studies have confirmed that both schizophrenia and diabetes underlie chronic low-grade inflammation with elevated IL-6 indicating that disturbances in immune response might be involved in concurrent onset of both conditions. Tulshi Chakraborty et al, in their study has mentioned that biomarkers like Autoantibodies, C-reactive protein, Plasminogen activator inhibitor (PAI-1), Plasminogen activator inhibitor (PAI-1), etc. are used for diagnosis; Adiponectin, microRNA, Acyl carnitine, Hemoglobin A1c, etc. are used for treatment, and 1,5-anhydroglucitol are used for both diagnosis and treatment of diabetes mellitus. 6. CONCLUSION Raised levels of Interleukin-6 shows progression of inflammatory mechanisms in diabetes patients. High pro-inflammatory marker IL-6 results in development of microvascular and macrovascular complications. In addition to periodic checking of HbA1c, it is paramount important to check the inflammatory status in diabetics to prevent diabetic complications. Anti-IL-6 drugs have been developed and already used for treatment of various diseases. This study provides valuable information to clinicians for developing clinical management protocols for patients with type 2 diabetes mellitus. 7. ACKNOWLEDGMENTS We express our sincere gratitude to all teaching and technical staff members of Department of Biochemistry, Department of Medicine Sree Balaji medical college and Hospital. 8. CONFLICT OF INTEREST No conflicts of interest are declared by the authors. 9. AUTHORS CONTRIBUTION STATEMENT Sample collection, processing was done by Dr. Mary Chandrika, discussion and write up done by Dr. V. S. KalaiSelvi.


