Interleukin-6 in impaired fasting glucose

Ashish Agarwal, Anupama Hegde*, Charu Yadav, Afzal Ahmad, Poornima A Manjrekar & Rukmini MS
Department of Biochemistry, Kasturba Medical College, MAHE, Manipal University, Mangalore -575 004, Karnataka, India

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Prediabetes is associated with the features of metabolic syndrome and inflammation contributing directly to the pathogenesis of cardiovascular disease (CVD). This study was conducted to explore the utility of interleukin-6 (IL-6) in determining the risk of CVD in prediabetes. It involves estimation of IL-6 & insulin along with its correlation with insulin, fasting plasma glucose (FPG), Insulin resistance (IR) and physical measurements. Eighty subjects were grouped into 40 prediabetics and 40 normoglycemic on the basis of FPG values. The mean insulin, IL-6, Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) and anthropometric measurements were found to be significantly higher ($P <0.05$) in prediabetes group. IL-6 had a significant correlation with fasting insulin ($r = -0.413$) and HOMA-IR ($r = -0.413$), but no correlation with FPG ($r = -0.227$) in the prediabetes group. IL-6 also showed a positive correlation with body mass index BMI ($r = -0.339$), waist circumference WC ($r = -0.484$) and waist-to-hip ratio WHR ($r = -0.430$). This study concludes that prediabetes is associated with inflammation, increasing the risk of CVD in these individuals.

**Keywords**: Cardiovascular disease risk, Inflammation, Prediabetes, Insulin resistance, Interleukin-6

Isolated impaired fasting glucose (IFG), isolated impaired glucose tolerance (IGT), combined IFG and IGT are all constituents of prediabetes. IFG is defined by an elevated FPG concentration (101-125 mg/dL), whereas IGT is defined by an elevated 2 h plasma glucose concentration (141-199 mg/dL) after a 75-g glucose load on the oral glucose tolerance test (OGTT) in the presence of an FPG concentration <126 mg/dL. Prediabetes is associated with features of metabolic syndrome, inflammation and microangiopathy.

Various pathophysiologic pathways are involved in the development of CVD during prediabetes by both glycemic and non-glycemic factors leading to vasculopathy. Hyperglycemia induces oxidative stress leading to endothelial dysfunction contributing to the development of CVD. It is also associated with a procoagulant and prothrombotic state contributing to macroangiopathy. Insulin resistance and impaired insulin sensitivity are nonglycemic mechanisms contributing to the pathogenesis of CVD. They reduce nitric oxide (NO) production causing vascular endothelial dysfunction with compensatory hyperinsulinemia leading to vasoconstriction that secondarily promotes atherosclerosis. CVD primarily involves inflammatory and innate immune mechanisms. IL-6, a major pro-inflammatory cytokine, has been implicated as a link between low-grade inflammation, atherosclerosis, and CVD. It induces the secretion of tissue factors and cellular adhesion molecules via synthesis and secretion of C-reactive protein (CRP) leading to stimulation of macrophages and lymphocytes which promotes accumulation of foam cells by secreting cytokines and growth factors.

IL-6 has also been implicated in the process of vasculogenesis by inducing the expression of vascular endothelial growth factor (VEGF), which in turn induces the proliferation of vascular endothelial cells. It also regulates metabolism particularly fat metabolism, hence been related to obesity, insulin resistance and type II diabetes mellitus. In view of the reasons described above, estimation of IL-6 as an indicator of CVD risk in pre-diabetes forms the basis of this study.

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*Correspondence:
E-mail: anupama.hegde@manipal.edu

**Abbreviations**: BMI, Body mass index; CRP, C-reactive protein; CVD, Cardiovascular disease; ELISA, Enzyme-linked immune sorbent assay; FPG, Fasting plasma glucose; HC, Hip circumference; HOMA-IR, Homeostasis Model Assessment-Insulin Resistance; IFG, Impaired fasting glucose; IGT, Impaired glucose tolerance; IL-6, Interleukin-6; IR, Insulin resistance; NO, Nitric oxide; OGTT, Oral glucose tolerance test; R, Pearson’s correlation; RSSDI, Research Society for Study of Diabetes in India; MU, Manipal University; SPSS, Statistical package for social science; VEGF, Vascular endothelial growth factor; WC, Waist circumference; WHR, Waist-to-hip ratio
Materials and Methods
Subject selection
A cross-sectional study was conducted over a period of one year (December 2013-December 2014) in a tertiary care hospital on subjects aged 25-45 years who came with a requisition for fasting plasma glucose (FPG) test. Of the total 200 members screened, 75 subjects were eliminated on the basis of exclusion criteria i.e. history of diabetes, endocrine disorders, kidney diseases, cardiac diseases, any infectious disease in the past two weeks and pregnancy. Based on FPG results, those with values ≥126mg/dL (45 subjects) were excluded. Further, FPG 101-125mg/dL was grouped as prediabetes and FPG 70-100mg/dL as normoglycemics (40 subjects each) (Fig. 1).

The study was carried out with the approval of the institutional Ethics Committee (IEC KMC MLR 09-13/164). After obtaining informed consent, the history of the subjects was taken through a structured interview and anthropometric measures were recorded for each subject. The height, weight, hip circumference (HC), waist circumference (WC) and BMI & WHR were calculated.

Sample Collection, Preservation, and Analysis
A blood sample was collected in plain vacutainer for IL-6 & insulin estimation. The serum obtained was stored at −20°C until analysis. The assays for IL-6 and insulin were carried out using solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle in ELx 800 by BIO TEK® instruments, Inc. using commercially available kits provided by Ray Biotech, Inc. and DRG International, Inc., respectively. IR was calculated using the HOMA-IR calculator (University of Oxford, website: http://www.dtu.ox.ac.uk/homacalculator/index.php).

Statistical Analysis
Statistical package for social science SPSS vers.16.0 was used and P <0.05 was considered significant. Comparison between the groups was done by independent sample ‘t’ test and correlation was done by Pearson’s correlation (r).

Results
Subjects were age-matched and mean FPG differed significantly between the groups as per selection criteria. The mean values of all the anthropometric measures were found to be significantly higher in prediabetes group. (Table 1). The mean serum IL-6, insulin, and HOMA-IR were also found to be significantly increased in them (Fig. 2).

IL-6 significantly correlated with insulin (r = −0.413) and HOMA-IR (r = −0.413), but not with FPG (r = −0.227) in prediabetes group (Fig. 3).

Fasting Insulin, HOMA-IR and IL-6 positively correlated with BMI, WC and WHR (P <0.05) (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prediabetes group (n =40)</th>
<th>Normoglycemics (n =40)</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.95 ± 6.08</td>
<td>36.05 ± 5.89</td>
<td>0.16</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>109.18 ± 7.51</td>
<td>92.98 ± 4.23</td>
<td>0.000*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.29 ± 1.38</td>
<td>22.81 ± 1.50</td>
<td>0.000*</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>99.10 ± 4.74</td>
<td>87.22 ± 7.44</td>
<td>0.000*</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>104.62 ± 3.45</td>
<td>102.53 ± 4.55</td>
<td>0.023*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94 ± 0.04</td>
<td>0.85 ± 0.05</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

[values are expressed as mean ± SD]
N – number of subjects, FPG - Fasting Plasma Glucose, BMI – Body Mass Index, WC – waist circumference, HC – hip circumference, WHR – waist-to-hip ratio, *P <0.05 was considered significant.
Discussion

The current study aimed to study the risk of CVD in prediabetes by determining serum IL-6, insulin, and HOMA-IR. At baseline, subjects with prediabetes had increased BMI, WC, HC and WHR in contrast to normoglycemics (P <0.05, Table 1) and also increased levels of IL-6, Insulin and HOMA-IR (P <0.05, Fig. 2).

Ferrannini\(^\text{15}\) found that prediabetes individuals with mild hyperglycemia have a higher BMI, WC, and WHR compared with normoglycemic subjects predisposing them to an increased risk of CVD.

Increased levels of fasting insulin and higher HOMA-IR in prediabetes group (Fig. 2) could be the cause for mild hyperglycemia observed in this group. The decrease in insulin production by the pancreas is normally preceded by IR where more insulin is required for the same amount of response which progress to type 2 diabetes with the minimal or complete termination of insulin production. IR is known to overlap several clinical conditions, including IFG, IGT and type 2 diabetes\(^\text{16}\). IFG individuals principally have hepatic insulin resistance with reduced early phase and normal late-phase insulin secretory response after standard glucose load test resulting in fasting hyperglycemia due to excessive fasting hepatic glucose production and an initial increase of plasma glucose in the 1 h with normal 2 h value. A strong correlation of fasting Insulin and HOMA-IR (Table 2) with anthropometric measurements (BMI, WC, and WHR) in prediabetes group indicates that overweight and central obesity has contributed to hyperinsulinemia and IR resulting in hyperglycemia\(^\text{17-19}\). IR also leads to CVD through various mechanisms. Hyperinsulinemia and

![Image showing comparison of Insulin, HOMA-IR and IL-6 between Prediabetes group and Normoglycemic group (*P <0.05)](image1)

![Image showing scatter plot correlation of IL-6 with FPG(A), Insulin(B) and HOMA-IR(C) (*P <0.05)](image2)

| Table 2—Correlation of Insulin, HOMA-IR, and IL-6 with Anthropometric Measurements |
|-------------------|---------------------|---------------------|---------------------|
| Parameter         | Insulin (μIU/mL)    | HOMA-IR             | IL-6 (pg/mL)        |
| BMI (Kg/m\(^2\))  | 0.446*              | 0.443*              | 0.339*              |
| WC (cm)           | 0.361*              | 0.359*              | 0.484*              |
| HC (cm)           | −0.292              | −0.300              | 0.141               |
| WHR               | 0.622*              | 0.625*              | 0.430*              |

[Values are expressed as Pearsons correlation]
BMI – Body Mass Index, WC – waist circumference, HC – hip circumference, WHR – waist-to-hip ratio, IL-6 – Interleukin-6, \(*P <0.05\) considered significant.
IR causes dyslipidemia, hypertension, increased clotting, endothelial dysfunction and stimulating division and migration of vascular smooth muscle cells.

Chronic inflammation has been suggested to play a causal role in endothelial dysfunction and atherosclerotic plaque formation contributing to the development of vascular complications in patients with diabetes. Sommer et al. found that hyperglycemia induces IL-6 production. Persistent hyperglycemia leads to the formation of advanced glycation end products resulting in the development of chronic inflammation. Elevated serum IL-6 concentration in the prediabetes group (Fig. 2) indicates the presence of ongoing inflammatory process in this group, but no correlation could be established between FPG and IL-6 (Fig. 3A). Hossain et al. reported IL-6 to correlate with glucose levels in prediabetes with IGT but not IFG. Correlation of IL-6 with Insulin and HOMA-IR (Fig. 3B & 3C) indicates that hyperinsulinemia and IR are associated with subclinical inflammation. According to Pickup and Crook hypothesis, stimuli such as over-nutrition would result in cytokine hypersecretion, mainly IL-6 eventually leading to IR which accelerates the process of atherosclerosis and CVD. IL-6 correlated with weight, BMI, WC and WHR in prediabetes subjects indicating that increased weight strongly contributes to the development of chronic inflammation (Table 2). In vitro study has demonstrated that adding the extract of adipocytes to human umbilical venous endothelial cells increases production of IL-6 by these cells. Previously documented findings indicate that IL-6 is produced by adipose tissue macrophages, which may have an important role in the development of obesity and insulin resistance. The mechanism has been attributed to impaired insulin sensitivity and insulin resistance in target tissues with increased lipolysis and decreased glucose uptake in the adipose tissue due to IL-6 induced reduction in tyrosine phosphorylation, and elevation of serine phosphorylation.

Conclusion

Prediabetes with IFG is associated with central adiposity, IR, and low-grade inflammation as measured by IL-6. Collective presence of pro-inflammatory status at the initial level of hyperglycemia predisposes the subjects to the risk of CVD.

References


