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ORIGINAL ARTICLE

Association of Interleukin-6 and Myeloperoxidase with Insulin Resistance in Impaired Fasting Glucose Subjects

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Abstract Impaired fasting glucose (IFG) is a high risk subclinical condition for the progression of type 2 diabetes mellitus and the hyperglycemia seen in this condition is because of the development of insulin resistance (IR). Obesity, inflammation, oxidative stress and many other factors have been implicated in development of IR in type 2 diabetes mellitus and its successive complications. Current study was aimed to ascertain the correlation of inflammation and oxidative stress markers [interleukin-6 (IL-6) and myeloperoxidase (MPO)] with IR in subjects with IFG. In this study, 80 subjects (40 IFG, 40 healthy controls) aged 25–45 years were selected based on their fasting plasma glucose (FPG) values and clinical history. Serum insulin, IL-6 and MPO were estimated by ELISA method and IR was calculated using Homeostatic Model Assessment Index 2 (HOMA 2) calculator. Pearson's correlation coefficient and independent sample '*t*' test were used for statistical analysis. IL-6 and MPO were found to be significantly elevated in IFG group and both correlates significantly with IR (r 0.413, r 0.645). Only MPO had significant correlation with FPG (r 0.388). In conclusion, the association of altered levels of IL-6 and MPO with IR are suggestive of a role of inflammation and oxidative stress in the initiation and progression of IR in individuals with IFG.

Keywords Impaired fasting glucose · Insulin resistance · Interleukin-6 · Myeloperoxidase

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Introduction

Spectrum of pre-clinical conditions like isolated impaired fasting glucose (IFG), isolated impaired glucose tolerance (IGT), combined IFG and IGT as well as high-risk glycosylated hemoglobin (HbA1c) constitute the definition of prediabetes [1, 2]. Insulin resistance (IR) and β-cell dysfunction involves the underlying pathophysiology of prediabetes and its subsequent progression to overt diabetes. [3] The mechanism responsible for IR has not been clearly established. Recent research shows that leptin, resistin, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) [4] and myeloperoxidase (MPO) [5] play a crucial role in the emergence of IR.

IL-6, a major proinflammatory cytokine produced mostly by adipocytes, has a controversial role in IR development [6]. Studies have reported equivocal results with some suggesting significant correlation of IL-6 with fasting plasma glucose (FPG) and insulin in IGT only and not in IFG [7, 8] while others could not establish any relationship between IL-6, FPG, insulin and IR [9]. Thus, relationship of IL-6 with IR remains ambiguous and had to be studied further.

MPO, an enzyme known for its bactericidal activity and oxidative stress, has also been associated in the development of IR [5]. Studies have observed a role of oxidative stress in the development of IR [10], their association with prediabetes [11] and obesity [12].

There is limited data relating inflammation [7–9] and oxidative stress [10–12] to IR in IFG [13]. Hence, in the present study we measured serum concentrations of IL-6 and MPO, to test the assumption that elevated levels of inflammatory and oxidative stress markers are linked with IR in individuals with IFG.

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Materials and Methods

Study Subjects

For this hospital based cross-sectional study a total of 300 subjects were screened over a period of 1 year (December 2013–December 2014) out of which 150 subjects were excluded as per the exclusion criteria which includes age <25 or >45 years, on treatment dyslipidemia or hypertension, history of diabetes, endocrine disorders, kidney diseases, cardiac diseases, any infectious disease in the past 2 weeks, pregnant or lactating women. Another 70 subjects were excluded out of these 150 subjects whose FPG was found to be ≥126 mg/dl as they were in the diabetes range. The remaining 80 subjects who met the inclusion criteria i.e. FPG of 101–125 or 70–100 mg/dl were selected and categorised into IFG (n = 40) and healthy controls (n = 40) respectively.

The study protocol was approved by the Ethical Committee of Manipal University (Protocol number IEC KMC MLR 09-13/164) and was in accordance with the principles outlined in the Declaration of Helsinki. All the subjects gave written informed consent before enrolment into the study.

Body weight was assessed using an adult balance with the individuals in standing position to the nearest 0.1 kg. Height was measured at the highest head point, with a stadiometer to the nearest 1.0 cm. Body mass index (BMI) was calculated using Quetlet's Index (weight (kg)/height $(m)^2$), expressed in kg/m^2.

Waist circumference (WC, to the nearest 1 cm) was measured using inelastic tape placed horizontally and tension free immediately over the skin at the midpoint between the last rib and the iliac crest, with measurements taken at the end of expiration. Hip circumference (cm) was measured taking as reference the largest circumference on the hip anatomy, and waist-to-hip ratio (WHR) was then calculated.

Blood Samples Collection and Measurement of Analytes

For the biochemical analysis, patients underwent peripheral venous blood collection from an antecubital vein after an overnight fasting. Immediately after, samples destined to the determination of general biochemical profile were processed according to standard laboratory techniques. Next, serum samples were aliquoted and stored at −20 °C until analysis.

FPG in plasma was measured with glucose oxidase–peroxidase (GOD–POD) enzymatic method by the Roche Hitachi cobas P800 autoanalyzer; intra-assay coefficients of variation (CV) was <2 %. The results were expressed in mg/dl. For quantitative insulin estimation in serum, a commercial ELISA kit was used (DRG International, Inc., USA), following the manufacturer's instructions in ELx 800 by BIO TEK® Instruments, Inc. A standard curve was used to determine insulin concentrations; intra-assay CVs was <2.6 %. The results were expressed in μIU/ml. The degree of insulin resistance was estimated using Homeostatic Model Assessment Index 2 (HOMA 2) calculator. High HOMA 2 values indicate a state of insulin resistance, while low HOMA 2 values are associated with better insulin sensitivity. The quantification of serum IL-6 and MPO levels was performed by ELISA reader (ELx 800 by BIO TEK® Instruments, Inc.), with commercial kits (IL-6 ELISA kit, RayBiotech, Inc., USA and MPO ELISA kit, Immunology Consultants Laboratory, Inc., USA respectively), according to the manufacturer's instructions. Independent standard curves were used to determine their concentrations; intra-assay CVs were <10 % for both the analytes. The results were expressed in pg/ml and ng/ml respectively.

Statistics

Data were analyzed using Statistical Package for Social Science (SPSS), version 16.00 (IBM SPSS statistics), and normality was tested by plotting histograms. As all the data presented here are normally distributed, Independent sample '*t*' test was used to compare means between the two groups and correlation was done by Pearson's correlation and represented as r. The results were considered significant if $p < 0.05$.

Results

Table 1 shows the baseline characteristics of the study subjects. The predominance of male patients can be seen in both groups. Age and height showed no considerable differences between the groups. In IFG individuals, weight, body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR) were increased in comparison to the controls.

The mean fasting glucose was expected to be significantly high in IFG group since it is the inclusion criteria (Table 2). Fasting insulin levels, IR (HOMA 2), IL-6 and MPO were also significantly increased in IFG subjects (Table 2).

When Pearson's correlation was applied for serum IL-6 and MPO versus FPG, fasting insulin and HOMA-2 values, significant positive correlation of both the markers was seen with fasting insulin (r 0.413, r 0.647) (Table 3) and

Table 1 Baseline characteristics of the IFG and healthy group

Variable	IFG group (n = 40)		Healthy controls (n = 40)		*p* value
Gender	Male (n/%)	female (n/ %)	Male (n/%)	female (n/%)	
	26/65	14/35	32/80	8/20	
Age (years)	37.95 ± 6.08 (0.96)		36.05 ± 5.89 (0.93)		NS
Height (cm)	158.22 ± 5.80 (0.91)		159.72 ± 8.09 (1.28)		NS
BMI (kg/m^2)	27.29 ± 1.38 (0.21)		22.81 ± 1.50 (0.23)		<0.001*
WC (cm)	99.10 ± 4.74 (0.75)		87.22 ± 7.44 (1.17)		<0.001*
HC (cm)	104.62 ± 3.45 (0.54)		102.53 ± 4.55 (0.71)		0.023*
WHR	0.94 ± 0.04 (0.006)		0.85 ± 0.05 (0.008)		<0.001*

Results are shown as mean ± SD (*SE* standard error of mean), *IFG* impaired fasting glucose, *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

Table 2 Comparison of FPG, insulin, HOMA 2, IL-6 and MPO between the two groups

Marker	IFG group	Healthy controls	*p* value
FPG (mg/dl)	109.18 ± 7.51 (1.18)	92.98 ± 4.23 (0.66)	<0.001
Insulin (μIU/ml)	27.86 ± 12.48 (1.97)	12.49 ± 3.55 (0.56)	<0.001
HOMA-2	3.62 ± 1.57 (0.24)	1.62 ± 0.43 (0.06)	<0.001
IL-6 (pg/ml)	66.29 ± 15.39 (2.43)	12.59 ± 2.69 (0.42)	<0.001
MPO (ng/ml)	67.46 ± 13.77 (2.17)	46.78 ± 9.93 (1.57)	<0.001

Results are shown as mean ± SD (*SE* standard error of mean), *IFG* impaired fasting glucose, *FPG* Fasting plasma glucose, *HOMA-2* homeostasis model assessment-2, *IL-6* interleukin 6, *MPO* Myeloperoxidase, $p < 0.05$ was considered significant

Table 3 Correlation of FPG, insulin and HOMA 2 with IL-6 and MPO in IFG

Parameter	IL6 (pg/ml)		MPO (ng/ml)	
	r value	*p* value	r value	*p* value
FPG (mg/dl)	0.227	0.158	0.388	0.013*
Fasting insulin (μIU/ml)	0.413	0.008*	0.645	<0.001*
HOMA-2	0.413	0.008*	0.647	<0.001*

FPG Fasting plasma glucose, *HOMA-2* homeostasis model assessment-2, *IL-6* interleukin 6, *MPO* Myeloperoxidase, *r* Pearsons correlation coefficient, * $p < 0.05$ was considered significant

HOMA-2 (r 0.413, r 0.645) (Table 3; Figs. 1, 2). Only MPO was found to be correlated significantly with FPG (r 0.388) (Table 3).

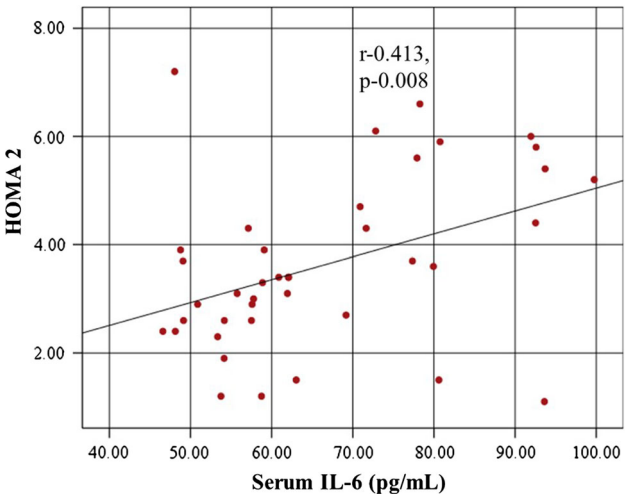

Fig. 1 *Scatter plot* showing correlation of HOMA 2 (IR) with serum IL-6 levels in IFG subjects. *HOMA 2* Homeostatic Model Assessment Index 2, *IL-6* Interleukin 6, r—Pearsons correlation, $p < 0.05$ was considered significant

Discussion

The present study was conducted to test the assumption that raised levels of inflammatory and oxidative stress markers are linked with IR in individuals with pre-diabetes i.e. IFG, for which serum IL-6 (inflammatory marker) and MPO (oxidative stress markers) were estimated in IFG subjects and healthy controls and correlated with FPG, fasting insulin and IR (HOMA-2). The baseline characteristics of the subjects were measured and documented (Table 1). The subjects did not show significant difference in their age and were therefore well matched.

Mean weight, BMI, WC, HC and WHR were all significantly increased in IFG as compared to normal healthy

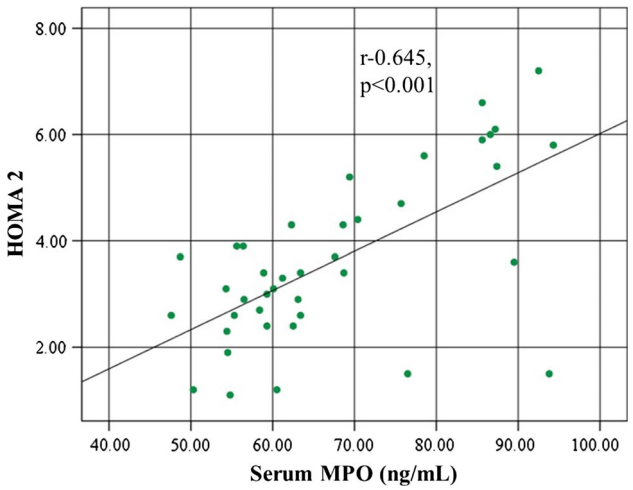

Fig. 2 *Scatter plot* showing correlation of HOMA 2 (IR) with serum MPO levels in IFG subjects. *HOMA 2* Homeostatic Model Assessment Index 2, *MPO* Myeloperoxidase, r—Pearsons correlation, $p < 0.05$ was considered significant

controls (Table 1). In this context, Ferrannini [14] suggested that prediabetes individuals have mild hyperglycemia, higher BMI, more central fat distribution and higher waist-to-hip ratio compared with normoglycemic subjects. All these parameters lead to the development of IR, therefore commonly present in prediabetes. [15] Furthermore, excess body fat promotes systemic oxidative stress and unbalanced production of adipokines, contributing to the pathogenesis of IR [16].

Increased levels of fasting insulin and higher HOMA-2 in IFG group (Table 2) could be the cause for mild hyperglycemia observed in this group. Progressive decline in insulin production by pancreatic β-cells is preceded by the emergence of IR, followed by gradual progression to type 2 diabetes where overproduction of insulin cannot be sustained by the pancreas [17]. Various mechanisms for the development of IR have been proposed involving IL-6 and MPO.

IL-6 stimulates the release of adrenocorticotropic hormone (ACTH) and growth hormone in the brain causing induction of IR [9]. In hepatocytes it suppresses tyrosine phosphorylation of insulin response element (IRS-1), mediated through hepatocyte-specific suppressor of cytokine signaling proteins (SOCS-3) which inhibit insulin-induced signaling resulting in IR. In adipocytes, IL-6 has been shown to induce the expression of adipocyte-specific SOCS-3 which inhibits insulin-induced glucose uptake in adipocytes. Another potential mediator of the inhibitory effect of IL-6 on insulin action in adipocytes is adiponectin, the expression of which is inhibited by IL-6 in 3T3-L1 adipocytes [18]. MPO on the other hand is not involved directly in the pathogenesis of IR, but indirectly through the reactive oxygen species (ROS) produced by it. These ROS causes IR through various mechanisms such as stimulation of stress signaling mitogen-activated protein kinases (MAPKs), triggering the production of proinflammatory cytokines (IL-6) and causing oxidative damage to critical macromolecules in insulin-sensitive tissues [19].

In this study, serum IL-6 and MPO, the biochemical measures of inflammation and oxidative stress were significantly increased in IFG group as compared to healthy controls (Table 2). Elevated serum IL-6 and MPO concentration in the IFG group indicates the presence of chronic inflammation and oxidative stress in this group. A study conducted by Marcovecchio et al. [20] reported persistent hyperglycemia as a positive effector for IL-6 production via advanced glycation end (AGE) products formation which are implicated in the development of chronic inflammation [21]. Gopaul et al. [11] also found that prediabetes state is linked with high circulatory levels of oxidative stress markers.

No correlation could be established between IL-6 and FPG (Table 3) in IFG group which is in agreement with the studies conducted by Hossain et al. [22] and Cardellini et al. [8]. Both these studies reported IL-6 correlation with FPG in IGT but not in IFG suggesting that the effect of plasma IL-6 concentration is more deleterious with acute hyperglycemia i.e. postprandial than continuous hyperglycemia i.e. fasting. A positive correlation of MPO with FPG (Table 3) in IFG group indicates the role of high glucose concentrations in stimulating vascular production of MPO and its reactive oxygen species causing deleterious effects [23].

Significant correlation of fasting insulin and HOMA 2 with IL-6 in IFG group (Table 3; Fig. 1) indicates that subclinical inflammation is associated with hyperinsulinemia and IR and can be involved in the development of the same. According to Pickup and Crook [24] hypothesis, stimuli such as over nutrition would result in hypersecretion of cytokine, mainly IL-6 and eventually lead to IR, diabetes and its complications [25]. Studies conducted by Konukoglu et al. [7] and Vazarova et al. [26] also reported significant correlation of IL-6 with insulin in prediabetes individuals and negative correlation to insulin action in Pima Indians respectively. But Cardellini et al. [8] and Choi et al. [9] could not establish any relationship between IL-6, insulin and IR.

In this study we also found positive correlation of fasting insulin and HOMA 2 with MPO in IFG group (Table 3; Fig. 2). This indicates ROS mediated oxidative stress alters the intracellular signaling pathway by inducing hyperinsulinemia and IR [27]. Oxidative stress damages mitochondria of pancreatic β-cells and markedly reduces insulin secretion [28]. Similar results were reported in an study conducted by Urkava et al. [10] where oxidative stress was found to activate the development of IR in men.

Andrade et al. [12] also reported increased MPO activity in obese women and Heinecke et al. [5] proposes MPO as a mediator of IR.

Limitations

The principal limitation relevant to the interpretation of these results is the use of cross-sectional data, which limits inferences about causal pathways. Further longitudinal studies in a larger sample can be taken up to ascertain the results and to establish a cause-effect relationship. Secondly, only fasting blood glucose measurement was the inclusion criteria which might have misclassified IFG. Therefore, studies can be taken up to include HbA1c also for further strengthening the inclusion criteria.

Conclusion

In conclusion, the association linking the altered levels of IL-6 and MPO with the levels of glycemic indices are suggestive of a role of inflammation and oxidative stress in the initiation and progression of IR in individuals with IFG which in turn could contribute to progression to overt diabetes.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Standard The study has been reviewed by an institutional review board, and has been performed in accordance with ethical standards laid down in an appropriate version of the 1964 of the Helsinki declaration.

Human and Animal Rights All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Informed Consent Informed consent was obtained from all patients for being included in the study.

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