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ELEVATED SERUM LIPOCALIN-2 IS A RISK FACTOR OF INSULIN RESISTANCE AND FOR THE PROGRESSION OF IMPAIRED GLUCOSE TO TYPE 2 DIABETES IN BANGLADESHI POPULATION

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Abstract

Background: Lipocalin-2 is a novel adipokine has effect on glucose metabolism. In this study, we investigate the association of serum Lipocalin-2 with insulin resistance in Bangladeshi population having impaired glucose.

Methods: We estimated serum Lipocalin-2 and insulin in 171 Bangladeshi people by ELISA. Anthropometric parameters, glucose, HbA1c and lipid profile parameters were also measured.

Result: Serum Lipocalin-2 was significantly elevated in the study subjects of impaired glucose tolerance and newly diagnosed type 2 diabetes compared to healthy control. In regression analysis, serum Lipocalin-2 concentration is independently and closely associated with the insulin resistance (HOMA-IR) in both IGT and NDD study groups (β = -0.249, p = 0.034, 95% CI -0.010 – 0.00 in IGT group and β = -0.237, p = 0.033, 95% CI -0.012 – 0.00 in NDD group) after adjustment of various confounding variables.

Conclusion: Our findings predict that elevated serum Lipocalin-2 seems to have a contribution to the pathophysiology of insulin resistance and progression of impaired glucose to type 2 diabetes.

Keywords: Lipocalin-2, impaired glucose tolerance, insulin resistance, type 2 diabetes, glucose metabolism, HbA1c, lipid profile.

Introduction

The excessive visceral obesity and obesity related risk factors are associated with the rising incidence of cardiovascular diseases and type 2 diabetes mellitus [2, 3]. It is prominent that the visceral obesity is quantified by the functions of

adipose tissue [4]. However, adipocytes and adipose tissue produce a wide range of hormones and cytokines involved in glucose metabolism (e.g. adiponectin, resistin), lipid metabolism (e.g. cholesteryl ester transfer protein, CETP), inflammation (e.g. TNF-a, IL-6), coagulation (PAI-1), blood pressure (e.g. angiotensinogen, angiotensin II), and feeding behaviour (leptin) thus affecting metabolism and function of many organs and tissues including muscle, liver, vasculature, and brain [5–7]. Type 2 diabetes is now generally accepted to be due to a combination of insulin resistance and relatively diminished insulin secretory function of pancreatic β-cells. β-cell dysfunction is the most important risk factor for type 2 diabetes as shown in normoglycemic subjects [8, 9]. In most studies low adiponectin and elevated levels of other adipocytokines (e.g. leptin, TNF-a, IL-6) are associated with an increased risk of diabetes. This presumably relates not only to their effects on insulin sensitivity but also to their effects in the pancreas leading to β-cell failure [10, 11]. Lipocalin-2, also known as neutrophil gelatinase-associated lipocalin or neu-related lipocalin, belongs to the lipocalin super family. It shows high affinity to several small hydrophobic ligands such as retinoid, fatty acids, pheromones, steroids and iron [12]. It is secreted and expressed in adipose tissue, kidney, lung, liver, brain, neutrophil as well as macrophage to mediate various biological functions and seems to affect glucose metabolism and insulin sensitivity with high level expression [13, 14]. In an animal model study showed that lipocalin-2 is regulated by obesity and promotes insulin resistance [15]. Furthermore, a human study reported that levels of lipocalin-2 were elevated in both circulation and adipose tissue which is observed among diabetic patients and this could be reversed by the insulin-sensitizing drug rosiglitazone [16]. However, a large scale population based study showed a relationship of lipocalin-2 with glucose metabolism, insulin resistance and chronic low grade systemic inflammation [17]. But it needs further investigation in another population to provide more data. Thus, for better evaluation of the role of lipocalin-2 in glucose metabolism and insulin resistance we have conducted an observational group comparing study in Bangladeshi population in which 171 subjects were recruited irrespectively of race and religion and we examined the distribution of serum lipocalin-2 in the study groups and its association with insulin resistance and other metabolic parameters.

Methods

Study population

The design of the study was an observational group comparing study in which subjects were selected purposively. Our study subjects were collected irrespectively of race, religion and socioeconomic status from the OPD of BIRDEM and

BIHS hospital, Dhaka, Bangladesh. All studied individuals were recruited with a stringent inclusion (age between 30-50 years, not exposed to insulin and anti-diabetic drugs) and exclusion (age <20, >50 years, serious comorbid diseases, exposed to drugs affect in glucose metabolism, pregnant and lactating mothers) criteria. A written consent and history of the subject was taken from all the participants with a predesigned history record form.

Data collection

A standardized questionnaire was designed by trained physicians to collect information such as age, sex, physical activity, socioeconomic status, smoking (yes/no), alcohol drinking (yes/no) and self reported diabetes, hypertension and dyslipidemia. Family history of diabetes, hypertension, liver diseases and kidney diseases were taken as yes or no. All study subjects were assessed after overnight fasting for 8 to 14 hours. The anthropometric measurements were carried out by trained technician including height, weight, waist circumference, hip circumference, percentages of body fat (calculated by fat monitor) and blood pressure. BMI was calculated as weight in kilograms divided by square of height in meters and categories as normal, overweight and obese according to the WHO criteria [1].

Laboratory measurements

Peripheral venous blood samples were collected. The fasting glucose, glucose 2 h after oral glucose tolerance test, total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol were measured on an automated analyzer (Hitachi 7080). HbA1c was measured by automated high performance liquid chromatography (Bio-Rad VARIANT Ⅱ). The study groups of healthy control, impaired glucose tolerance and newly diagnosed type 2 diabetes were defined by ADA criteria 2010. A fasting glucose level lower than 5.6 mmol/l and a 2 h OGTT plasma glucose level below 7.8 mmol/l were defined as healthy control. Impaired glucose tolerance (IGT) group was defined as fasting glucose 5.6 to 6.9 mmol/l and 2 h OGTT plasma glucose 7.8 to 11.0 mmol/l and newly diagnosed type 2 diabetes group was defined as fasting glucose ≥7.0 mmol/l and 2 h OGTT plasma glucose ≥11.1 mmol/l.

Measurements of serum Insulin and Lipocalin-2

Serum insulin was estimated in duplicate by ELISA (EIA-2935, DRG in USA) as recommended by the manufacturer and serum lipocalin-2 was also estimated in duplicate by ELISA (DLCN20, R&D systems in USA) as recommended by the manufacturer. Insulin resistance was estimated using homeostasis model assessment index-insulin resistance (HOMA-IR).

Statistical data analysis

Statistical analysis was carried out by using the software of statistical package for social science (SPSS) version 15.

Results

Characteristics of the study subjects

In our study, a total of 171 subjects were recruited. Among those 35 with healthy control, 56 with impaired glucose tolerance (IGT) and 72 with newly diagnosed type 2 diabetes (NDD). There was no significant difference in anthropometric parameters (age, BMI, waist circumference, waist hip ratio, body fat percentages, systolic and diastolic blood pressure), cholesterol, HDL and LDL cholesterol and triglyceride between the study groups. The healthy control and newly diagnosed type 2 diabetes groups had the most favorable and unfavorable metabolic parameters respectively.

Distribution of serum Lipocalin-2 among the study groups

Serum lipocalin-2 concentrations were significantly higher in the study groups of impaired glucose tolerance (IGT) and newly diagnosed type 2 diabetes (NDD) compared with the group of healthy control (92.05 and 97.35 ng/ml vs 39.99 ng/ml, respectively, all $p < 0.001$, Figure-1).

Determinants of insulin resistance (HOMA-IR)

In this study, Body Mass Index (BMI) is independently associated with the insulin resistance ($\beta = 0.385$, $p<0.001$, 95% CI 0.044 – 0.140 in IGT group and $\beta = 0.431$, $p<0.001$, 95% CI 0.071 – 0.179 in NDD group) where as HDL cholesterol is independently associated with the group of impaired glucose tolerance ($\beta = -0.206$, $p = 0.045$, 95% CI -0.045 – 0.00) but not in NDD group (Table-2). In regression analysis, serum Lipocalin-2 concentration is independently and closely associated with the insulin resistance ($\beta = -0.249$, $p = 0.034$, 95% CI -0.010 – 0.00 in IGT group and $\beta = -0.237$, $p = 0.033$, 95% CI -0.012 – 0.00 in NDD group) after adjustment of various confounding variables.

Table-1: Characteristics of the study population.

Characteristics	Control	IGT		NDD	
	Mean±SD	Mean±SD	P value	Mean±SD	P value
n	35	56		72	
Age (years)	40.66±9.90	42.82±9.20	0.380	45.94±8.79	0.524
BMI (kg/m^2)	22.11±3.51	25.52±4.04	0.454	25.86±3.78	0.736
Waist (cm)	88.29±7.74	90.25±8.39	0.544	91.41±8.27	0.940
WHR	0.92±0.04	0.93±0.05	0.386	0.94±0.05	0.159

Body fat (%)	30.50±5.34	33.63±10.00	0.502	31.86±6.25	0.613
Systolic blood pressure (mmHg)	110±15	114±13	0.436	115±15	0.720
Diastolic blood pressure (mmHg)	75±10	76±10	0.725	77±10	0.711
Fasting blood sugar (mmol/L)	5.16±0.33	5.56±0.33	<0.001	7.32±1.40	<0.001
Postprandial blood sugar (mmol/L)	6.47±0.87	8.72±1.10	<0.001	12.73±3.17	<0.001
HBA1c (%)	5.59±0.46	5.78±0.59	0.113	6.67±0.94	<0.001
Cholesterol (mg/dl)	190.34±41.10	193.22±39.35	0.719	201.34±46.93	0.576
HDL cholesterol (mg/dl)	40.34±9.14	39.54±7.87	0.668	39.13±9.72	0.529
LDL cholesterol (mg/dl)	120.66±42.87	121.03±34.28	0.942	125.52±34.45	0.644
Triglycerides (mg/dl)	151.40±58.41	164.07±90.29	0.411	197.03±100.24	0.004
Fasting Insulin (µIU/ml)	16.07±7.27	16.62±7.57	0.730	18.59±9.92	0.143
Insulin after glucose (µIU/ml)	68.01±40.60	74.97±45.38	0.444	83.85±44.92	0.072
Insulin secretion (HOMA%B)	147.06±59.09	129.03±40.48	0.116	88.24±32.11	<0.001
Insulin sensitivity (HOMA%S)	58.11±26.81	54.55±24.33	0.522	45.59±17.20	0.015
Insulin resistance (HOMA-IR)	2.04±0.89	2.19±0.94	0.467	2.55±1.12	0.015
Lipocalin-2 (ng/ml)	39.99±20.58	92.05±51.17	<0.001	97.35±59.34	<0.001

Data are expressed as Mean (±SD); n, number of subjects; level of significance at p<0.05

Table-2: Linear regression analysis.

Variables	IGT				NDD			
	β	*p* value	95% CI		β	*p* value	95% CI	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
(Constant)		0.181	-0.57	2.98		0.856	-1.724	2.071
Age (years)	-0.152	0.147	-0.035	0.005	-0.073	0.449	-0.032	0.014

Sex group (male = 1, female = 2)	0.057	0.611	-0.301	0.509	-0.085	0.407	-0.630	0.258
Body mass index (kg/m^2)	0.385	<0.001	0.044	0.140	0.431	<0.001	0.071	0.179
HDL-cholesterol (mg/dl)	-0.206	0.045	-0.045	0.000	-0.108	0.256	-0.033	0.009
Lipocalin-2 (ng/dl)	-0.249	0.034	-0.010	0.000	-0.237	0.033	-0.012	0.000

Dependable variable: HOMA-IR; Adjusted $R^2 = 0.152$; the level of significance at $p<0.05$; β, regression coefficient; CI, confidence interval.

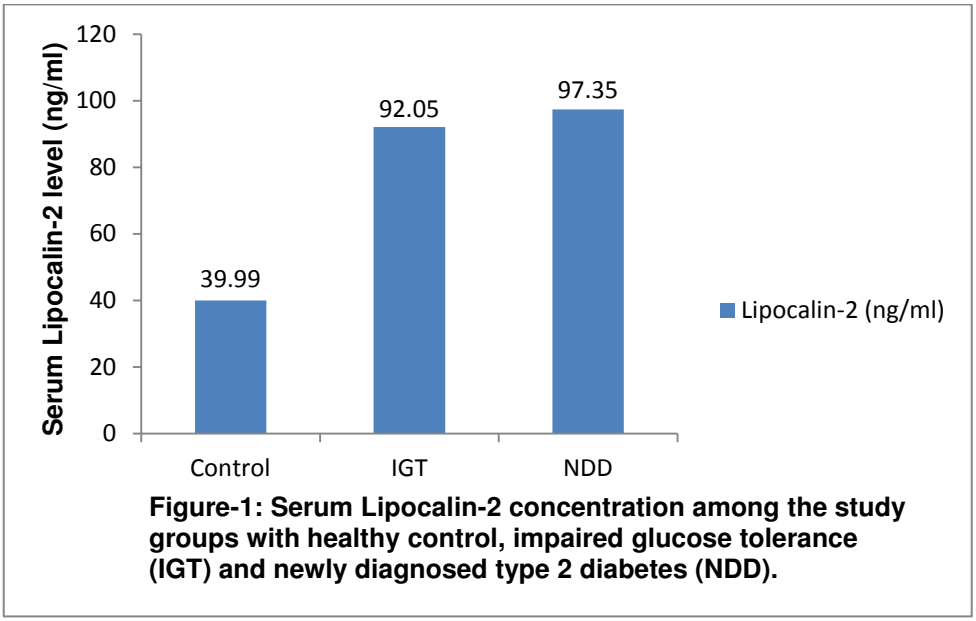

Figure-1: Serum Lipocalin-2 concentration among the study groups with healthy control, impaired glucose tolerance (IGT) and newly diagnosed type 2 diabetes (NDD).

Discussion

In this study, we found a close association of elevated serum Lipocalin-2 with insulin resistance in impaired glucose tolerance and type 2 diabetes in Bangladeshi population. Moreover, this association is independent of potential confounders.

Both insulin resistance and β-cell dysfunction occurs during the development of type 2 diabetes mellitus (T2DM), but controversy exists about which lesion is initiated first. Based on longitudinal studies in the Pima Indians, a population with the world's highest reported prevalence of T2DM, a two-step model for development of the disease is proposed. The first step is transition from normal to impaired glucose tolerance (IGT) for which insulin resistance is the main determinant, and the second step is worsening from impaired glucose tolerance (IGT) to type 2 diabetes in which β-cell dysfunction plays a critical role. This hypothesis is consistent with findings from other ethnic groups from many parts of the world [18]. However, several risk factors exist in the development of type 2 diabetes, among them obesity is the

most prominent. It is a consequence of excess accumulation of visceral fat which has been shown to be associated with adipose tissue inflammation and insulin resistance [19]. The present study found an association of BMI with insulin resistance both in impaired glucose tolerance and type 2 diabetes which is supported by several lines of studies. Firstly, a population based study found an association of body mass index with insulin resistance and pancreatic β-cell function in Korean patients with new- onset type 2 diabetes [20] and secondly, another study proposed arelationship of body mass index and insulin resistance to cardiovascular risk factors, inflammatory factors, and oxidative stress during adolescence [21]. Moreover, Serum Lipocalin-2 has been reported to be elevated in various conditions such as metabolic syndrome, cardiovascular diseases, obesity, type 2 diabetes and polycystic ovary syndrome [22, 23, and 17]. In this study, we also observed serum Lipocalin-2 is elevated both in impaired glucose tolerance and type 2 diabetes. Hence, we propose that elevated serum Lipocalin-2 might be a contributing factor of glucose metabolism. This hypothesis is supported by several lines of studies. First, a study investigated both in ex vivo and in vivo that Lipocalin-2 is up regulated by insulin via phosphatidylinositol 3-kinase and mitogen-activated protein kinase signaling pathways. [24] and secondly, another study on both human and animal model demonstrated that circulating Lipocalin-2 and its expression in adipose tissue and liver is significantly high [16]. Pancreatic β-cell dysfunction and insulin resistance are hallmarks of type 2 diabetes. In regression analysis, we found that elevated serum Lipocalin-2 and insulin resistance are closely associated in impaired glucose tolerance as well as newly diagnosed type 2 diabetes. As a consequence, the present study predicted that elevated serum Lipocalin-2 has a contribution to the pathogenesis of insulin resistance and progression of impaired glucose to type 2 diabetes, though the exact mechanism remain unclear. This prediction is in lined with several studies. Firstly, a large scale population based studies showed a close association of elevated serum Lipocalin-2 with impaired glucose metabolism and type 2 diabetes [17]. Secondly, an animal model study suggested that deficiency of Lipocalin-2 has a protection from obesity induced insulin resistance [25]. To our knowledge, this is the first study to investigate the association of serum Lipocalin-2 with insulin resistance and progression of impaired glucose to type 2 diabetes in Bangladeshi population. However, there are still some limitations in this work. First, small number of sample volume. Second, it was an observational group comparing study.

Conclusion: In conclusion, our study suggested that elevated serum Lipocalin-2 is associated with the pathophysiology of insulin resistance and seems to have a contribution to the progression of impaired glucose to type 2diabetes.

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