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Increased Fasting Plasma Retinol-binding Protein-4 (Rbp4) Associates With Impaired Glucose Tolerant Subjects Visited in a Tertiary Hospital of Bangadesh

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Abstract: *Animal studies have been suggested that Retinol-binding Protein-4 (RBP4) associates in the development of type 2 diabetes (T2D) but human studies are still controversial. Therefore, the present study aims to investigate plasma RBP4 in subjects with Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT) whether its changes commences before the onset of T2D. This study has investigated 13 IFG, 43 IGT and 16 IFG-IGT along with 50 healthy subjects. Fasting insulin and RBP4 were measured using Enzyme-Linked Immunosorbent Assay (ELISA) technique. Insulin sensitivity (HOMA %S) and insulin secretory capacity (HOMA %B) were estimated using fasting plasma glucose and fasting insulin values through HOMA-CIGMA software. Fasting plasmaRBP4 (ng/ml) level was significantly higher in IGT and IFG-IGT subjects compared to controls. In binary logistic regression analysis RBP4 was found to be significantly associated with IGT (B=0.123, p=0.001) and IFG-IGT subject (B=0.146, p=0.012). RBP4 was found to be significantly correlated with thewaist-hip ratio (r=0.323, p=0.035), fasting insulin (r=0.338, p=0.027) and HOMA-IR (r=0.336, p=0.037) in IGT subjects; after adjusted the effects of age and BMI, RBP4 has still associated with IR in IGT (β=0.400, p=0.009). The above results could be concluded as a) RBP4 has been increased in IGT and IFG-IGT subjects; b) RBP4 is positively associated with insulin resistance (IR) in IGT subjects.*

Keywords: *Impaired Fasting Glucose, Impaired Glucose Tolerance, PlasmaRBP4*

Introduction

Obesity has been suggested to be strongly associated with inflammation and the development of insulin resistance and type 2 diabetes.[1-3] Adipose tissue may be considered as an endocrine organ that secretes many adipocytokine hormones (such as leptin, tumor necrosis factor-α, interleukin-6, and adiponectin) that modulate the action of insulin in other tissues.[4] Moreover, retinol-binding protein-4 (RBP4), a fat-derived adipokine that specifically binds to retinol[2], has been reported to provide a link between obesity and insulin resistance.[5] The RBP4 gene which locates on chromosome 10 (10q23.33) encodes a protein of 201 amino acids, near the region of the gene have been found to be linked to increased fasting glucose levels in European Caucasians and to type 2 diabetes mellitus (T2D) in Mexican–Americans.[6,7] Liver and adipose tissue are

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major organs for the expression of RBP4.[8] The role of RBP4 in obesity and insulin resistance was first discovered by Barbara Kahn's group where it has been found that mice with an adipose-specific knockout of GLUT4 gene developed insulin resistance in muscles and liver.[9] Over expression of GLUT4 in mice tissues, specially in adipose tissue, have shown an increased potential for glucose clearance.[10] An adipocyte-derived factor, RBP4, has been suggested to modulate insulin sensitivity through retinol-dependent or retinol-independent pathway on muscle and/or liver tissues of experimental animals.[5,9] Rosiglitazone (a peroxisome proliferator–activated receptor-γ agonist) was found to decreased circulating levels of RBP4 in experimental mice lacking GLUT4 and this experiment have also shown reduced insulin resistance. It has been documented that increased levels of blood RBP4 leads to glucose intolerance, whereas increased insulin sensitivity has been found in mice with knock out of the RBP4 gene. High-fat diet-induced insulin resistance has been found decreased infenretinide (which facilitates the excretion of RBP4 into urine) treated mice.[5]In skeletal muscle, RBP4 increases insulin resistance by inhibiting both insulin receptor substrate-1 phosphorylation and phosphatidylinositol-3-kinase activation, while increasing hepatic glucose production by increasing PEPCK expression.[5]

In 1997 and 2003, the Expert Committee on Diagnosis and Classification of Diabetes Mellitus[11,12] recognized an intermediate group of individuals whose glucose levels do not meet criteria for diabetes, yet are higher than those considered normal which is again supported by ADA in 2011.[13] These people were defined as having impaired fasting glucose (IFG) [fasting plasma glucose (FPG) levels 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L)], or impaired glucose tolerance (IGT) [2-h values in the oral glucose tolerance test (OGTT) of 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L)].Plasma RBP4 levels are increased in subjects with IGT,T2D,and inversely correlated with insulin sensitivity in non-diabetic subjects with a family history of T2D.[14-16] Circulating RBP4 levels have been correlated with the degree of insulin resistance in these subjects and the relationship is independent of obesity.[14]

In a study on children, RBP4 was found correlated with adiposity and insulin resistance in obese children, but it was not involved in the insulin resistance occurring during puberty.[17]In several studies, circulating RBP4 levels also did notcorrelate with insulin resistance, impaired glucose tolerance, T2D, or altered insulin secretion.[18-22]So, racial variation may have important roles for the modulation of insulin sensitivity by RBP4. Therefore, the present study has been designed to investigate the RBP4 in IFG and IGT subjects of both fasting and postprandial states to observe whether itinitiates modulation of insulin sensitivity before the onset of diabetes.

Materials and Methods

Study Subjects

A total of seventy four pre-diabetic (14 IFG, 43 IGT and 17 IFG-IGT) subjects irrespective of race, religion and socioeconomic status were recruited from the Out-Patient Department (OPD) of BIRDEM Hospital, Dhaka. Subjects were considered as IFG, IGT or IFG-IGT as WHO guidelines[23]. Along with pre-diabetic subjects, fifty age, sex and BMI matched healthy subjects without family history of diabetes were also recruited as Controls.

Sample Collection

In this study the agreed volunteers were requested to come on a prefixed convenient morning after an overnight fast (eight to ten hours). After taken written consent, 5 ml of fasting blood sample was drawn by venepuncture in EDTA containing tubes, subjects were then given 75g of glucose dissolved in 250 ml of water and requested to drink within 5 minutes.Two hours after glucose solution intake 5 ml of blood was taken again by venepuncture

in EDTA containing tubes. After 15 minutes of collection blood samples were centrifuged for 10 minutes at 2500 rpm to obtain plasma and were aliquot to keep frozen at -40°C for biochemical analysis.

Anthropometric and Biochemical Measurements

Weight (kg), height (cm), waist circumference (cm) and hip circumference (cm) were measured using standard technique; BMI (Body Mass Index) was calculated using standard formula (weight in kg/height in meter2).Plasma glucose was measured using glucose-oxidase method and lipid profile was measured by enzymatic endpoint method (Randox Laboratories, UK). Insulin (Linco Research, USA) and RBP4 (ALPCO diagnostics, USA) were measured using enzyme linked immunosorbent assay (ELISA). Insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were determined using fasting glucose and fasting insulin by homeostasis model assessment (HOMA) using HOMA-Sigma software[24].

Statistical Analysis

Data were expressed as mean ±SD. Student's t-test, Pearson's correlation analyses, binary logistic regression analysis and multiple linear regression analyses were performed using SPSS software (SPSS, Chicago, IL) for Windows version 12. P-values <0.05 were considered statistically significant.

RESULTS

Anthropometric and Biochemical Parameters

Mean age, BMI, waist-hip ratio, waist-height ratio and blood pressure of the IFG, IGT and IFG-IGT subjects were not significantly different compared to the healthy control subjects (table 1). Fasting insulin levels were significantly higher in IGT (p=0.002) and IFG-IGT (p=0.017) subjects compared to the Controls. Mean TG level was significantly higher in IFG (p=0.002), IGT (p=0.001) and IFG-IGT (p=0.013) subjects compared to control subjects.

HOMA%B, HOMA%S and HOMA IR

Insulin secretory capacity which is calculated as HOMA%B was significantly lower in IFG (p=0.0001) and IFG-IGT (p=0.001) subjects compared to control subjects. Insulin sensitivity which is calculated as HOMA%S was significantly lower in IGT (p=0.001) and IFG-IGT (p=0.001) subjects compared to control subjects (table 1). Insulin resistance which is calculated as HOMA IR was also found significantly higher in IFG (p=0.031), IGT (p=0.001) and IFG-IGT (p=0.002) subjects compared to control subjects (table 1).

Table 1: Anthropometric characteristics of the study subjects

Parameters	Controls (n=50)	IFG (n=14)	IGT (n=43)	IFG-IGT (n=17)	p-values		
					Control vs. IFG	Control vs. IGT	Control vs. IFG-IGT
Age, yrs	42±6	43±7	41±5	43±8	0.107	0.868	0.454
BMI, kg/m^2	24.7±4.0	24.5±3.2	25.4±4.5	26.0±5.3	0.971	0.349	0.169
WHR	0.89±0.05	0.93±0.04	0.92±0.06	0.90±0.11	0.059	0.244	0.875
WHtR	0.54±0.052	0.56±0.06	0.56±0.05	0.57±0.12	0.141	0.069	0.448
SBP, mm-Hg	109±13	112±10	114±13	111±12	0.551	0.061	0.549
DBP, mm-Hg	79±9	77±8	75±8	74±7	0.081	0.159	0.341
FPG, mmol/l	5.2±0.3	6.4±0.3	5.4±0.5	6.5±0.4	0.001	0.621	0.001
PPPG, mmol/l	6.1±1.1	6.6±1.0	8.9±0.7	9.6±0.7	0.232	0.001	0.001
Insulin, µIU/ml	9.5±2.5	9.3±1.2	13±4.5	12.0±2.9	0.5478	0.002	0.016
TG, mg/dl	120±29	189±79	185±86	201±88	0.002	0.001	0.013
Chol, mg/dl	203±28	209±83	211±48	199±34	0.410	0.431	0.497
LDL, mg/dl	141±29	139±78	133±41	127±26	0.652	0.691	0.278
HDL, mg/dl	33±10	36±8	30±7	34±14	0.887	0.009	0.749
HOMA %B	110±25	71±9	131±39	81±16	0.001	0.019	0.001
HOMA %S	86±17	81±13	67±16	63±18	0.647	0.001	0.001
HOMA-IR	1.20±0.18	1.35±0.21	1.70±0.56	1.69±0.42	0.031	0.001	0.002
RBP4, ng/ml	31±7	32±10	37±9	34±9	0.523	0.001	0.011

BMI=body mass index; WHR=waist-hip ratio; WHtR=waist-height ratio; SBP=systolic blood pressure; DBP=-diastolic blood pressure; FPG=fasting plasma glucose; PPPG=postprandial (2hrs after 75g glucose intake) plasma glucose; TG=triglyceride; Chol=plasma cholesterol; HOMA%S=insulin sensitivity; HOMA%B=insulin secretory capacity; HOMA-IR=Insulin resistance using HOMA method; RBP4=plasma retinol-binding protein-4.

Plasma RBP4 among the study Subjects

Fasting plasmaRBP4 (ng/ml) level was significantly higher in IGT (37±9, p=0.001) and IFG-IGT (34±9, p=0.011) subjects compared to control (31±7) subjects (table 1). In binary logistic regression analysis, when control group was taken as reference and the effects of age, BMI and waist-height ratios (WHtR) were justified, plasmaRBP4 have shown significantly associated with IGT (B=0.123, p=0.001) and IFG-IGT (B=0.146, p=0.012) groups (table 2).

Table 2: Binary logistic regression analysis taking Control as reference group

Variables	Control vs. IFG			Control vs. IGT			Control vs. IFG-IGT		
	Beta	S.E.	p-value	Beta	S.E.	p-value	Beta	S.E.	p-value
Age	0.03	0.048	0.531	-0.009	0.040	0.814	0.060	0.055	0.278
BMI	-0.131	0.122	0.282	-0.095	0.080	0.234	0.143	0.102	0.161
WHtR	10.4	7.116	0.143	10.217	5.427	0.060	-2.679	7.125	0.707
RBP4	0.042	0.040	0.300	0.123	0.036	0.001	0.146	0.058	0.012
Constant	-6.369	3.339	0.056	-7.162	2.839	0.012	-10.827	4.693	0.021

Spearman Correlation and Multiple Linear Regression Analysis

In Spearman correlation analysis, RBP4 was found to be positively associated with both WHR (r=0.323, p=0.035), fasting insulin (r=0.338, p=0.027) and IR (r=0.336, p=0.037) in IGT subjects (table 3). Association of RBP4 with IR remains significant in multiple linear regression analysis (β=0.400, p=0.009) when the effects of age and BMI were adjusted (table 4).

Table 3: Correlation of RBP4 with WHR, insulin and insulin resistance (HOMA-IR)

Group	RBP4 vs. WHR		RBP4 vs. Insulin		RBP4 vs. IR	
	r	p	r	P	r	p
Control	0.02	0.893	0.068	0.64	0.132	0.416
IFG	0.109	0.677	0.310	0.456	0.072	0.878
IGT	0.323	0.035	0.338	0.027	0.336	0.037
IFG-IGT	-0.134	0.648	-0.077	0.812	-0.199	0.535

r, correlation co-efficient; p, value of significance

Table 4: Multiple linear regression analysis of RBP4 adjusted with age and BMI

RBP4*Vs*	Control		IFG		IGT		IFG-IGT	
	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value
Constant		0.007	-0.245	0.747		0.05		0.002
Age	0.04	0.784	0.365	0.768	-0.199	0.163	-0.243	0.395
BMI	0.095	0.519	0.248	0.664	0.285	0.056	-0.737	0.04
HOMA-IR	0.146	0.313		0.634	0.400	**0.009**	-0.498	0.133

RBP4 Quartiles and other Parameters

To examine the association between plasma RBP4 concentrations and other parameters related to insulin resistance, we divided our study subjects into plasma RBP4 concentration quartiles (Table 5). The proportion of IGT and IFG-IGT subjects increased on increasing RBP4 concentration. Also, the female proportion, fasting insulin and post-challenge plasma glucose levels, triglyceride levels, and HOMA-IR values have shown increasing tendency on increasing RBP4 (table 5).

Table 5: Clinical characteristics of the study subjects according to the quartiles of plasma RBP4 concentrations

Variables	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	Trends
N (%)	31 (100)	31 (100)	31 (100)	31 (100)	
Gender, M/F	14/17	18/13	16/15	12/19	
Age, yrs	42±5	42±6	43±7	42±7	
BMI (kg/m^2)	24.5±3.8	24.7±4.1	25.4±4.6	25.1±5.0	
RBP4, median (range)	23.9 (17.4-27.7)	30.1 (27.9-32.2)	35.4 (32.7-37.6)	43.3 (37.9-58.8)	
NGT, n (%)	16 (32)	18 (36)	9 (18)	7 (14)	
IFG, n (%)	9 (52.9)	1 (5.9)	0	7 (41.2)	χ^2=30.9,
IGT, n (%)	5 (11.6)	9 (20.9)	17 (39.5)	12 (27.9)	p=0.0001
IFG-IGT, n (%)	1 (7.1)	3 (21.4)	5 (35.7)	5 (35.7)	
FSG, mmol/l	5.6±0.6	5.3±0.6	5.3±0.6	5.6±0.7	
PPSG, mmol/l	6.5±1.8	7.2 ±1.9	8.7±1.8**	7.9±1.9**	
Insulin, µIU/ml	9.7±2.1	10.6±2.6	11.4±3.1*	12.5±5.1	
HOMA-IR	1.28±0.27	1.43±0.38	1.71±1.11**	1.54±0.63*	
TG, mg/dl	142±57	156±105	158±76	185±73*	

Discussion

The Expert Committee of World Health Organization on Diagnosis and Classification of Diabetes Mellitus recognized an intermediate group as pre-diabetes which is defined as having impaired fasting plasma glucose (IFG) (fasting plasma glucose levels 5.6 mmol/L to 6.9 mmol/L), or impaired glucose tolerance (IGT) (plasma glucose concentration of2-hrs after 75g glucose intake7.8 mmol/L to 11.0 mmol/L].11 In the present study the fasting glucose levels (mmol/L) of IFG and IGT subjects were 6.4±0.3 and 5.4±0.5 whereas the values 2hrs after 75g glucose intake were 6.6±1.0 and 8.9±0.7.

RBP4 is an adipokine consistently associated with adiposity and insulin resistance in animal models.[5,9] It was also considered a promising adipokine in humans possibly linking to adiposity, insulin resistance, T2D, and certain components of the metabolic syndrome.[16,17,25] However, in severalclinical studies, associations and/or causality ofobserved RBP4 expression changes with these statescould not be found.[19,20,26]

In this study, we found that plasma RBP4 levels, measured by ELISA, are elevated in subjects with IGT and IFG-IGT subjects. It was interesting to note that RBP4 was abundant in human plasma, which is higher than the Korean population. RBP4 levels in our IGT subjects have also shown higher values than the Korean population.[16] Although the difference of RBP4 in normal (median, range: 18.1, 9.3-30.5) and IGT (median, range: 18.9, 11.2-45.8) group of Korean population[16]was significant but the absolute difference is very little as we have found in our normal (median, range: 30.1, 17.4-41.5), IFG (median, range: 27.5, 19.4-51.2), IGT (median, range: 35.4, 20.2-58.8) and IFG-IGT (median, range: 34.8, 18.4-44.8) subjects (median, range values of RBP4 have not shown in the table).The discripency between Korean and Bangladeshi population may be the difference origin of the reagents or may be the difference origin of the population.

In relation to the previous finding that obese subjects have higher plasma RBP4 levels than lean subjects[5], we found positive correlation ofplasma RBP4 levels with a obesity indicatorWHR (waist-hip ratio) in IGT subjects but not with BMI or percent body fat. However, ob/ob mice were found to have a 13-fold higher

plasma RBP4 level than control mice and, according to Yang et al.[5], obese human subjects with elevated plasma RBP4 levels had unequivocally higher BMIs than lean control subjects.Although we failed but lipid parameters of Augsburg population have shown correlated with RBP4 along with other metabolic risk factors like BMI, waist circumference and hypertension.[27]

Previous study[28] have shown that IGT subjects of Bangladeshi origin are associated with insulin resistance and in this study we have found that increased RBP4 in IGT subjects are independenly associated with insulin resistance (HOMA-IR) in multivariate regression analysis.

In a cross-sectional study conducted in Chinese adults aged >40 years, it was found that increased RBP4 levels increased the risk for hyperglycemia, including impaired glucose regulation and newly diagnosed type 2 diabetes, even after adjustment for a number of confounders.[29] An another previous study20have also been reported that plasma RBP4 levels were elevated in subjects with IGT or type 2 diabetes and that RBP4 was related to various clinical parameters known to be associated with insulin resistance.

Two hr.post-challengeplasma glucose levels were found to increase with plasma RBP4 quartile. Yang et al. initially reported that obesity, but not hyperglycemia is an important determinator of circulating RBP4 levels.[5] However, they could not examine the quantitative relation between plasma RBP4 and blood glucose levels. The possible mechanism underlying increased post-challenge plasma glucose levels in su bjects with higher plasma RBP4 levels probably concerns increased insulin resistance (HOMA-IR) with increasing circulating RBP4.

Conclusions

RBP4 has been described as an adipokine contributes to insulin resistance and diabetes in the experimental mouse model. In addition to the liver, RBP4 is also secreted by adipocytes of the fat tissue in a smaller portion and acts as a signal to surrounding cells, when there is a decrease in plasma glucose concentration. It is suspected that an elevated level of RBP4 attracts macrophages to the fat tissue, causes local inflammation, and leads to insulin resistance. In this study, itis first time we have measured RBP4 in Bangladeshi pre-diabetes subjects and it has been found that i) RBP4 was found to be higher in Bangladeshi IGT and IFG-IGT subjects compared to nondiabetic control subjects, ii) RBP4 has been positively associated with waist-hip ratio, fasting plasma insulin levels and HOMA IR in IGT subjects, iii) Frequency of IGT and IFG-IGT has been increasing with RBP4 increasing.

Limitation of the study: One of the major limitations of the study is a smaller number of patients recruited.It would be better if we could analyze the polymorphism in RBP4 gene for its involvement in the formation of insulin resistance and diabetes.

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Conflict of interest: There is no conflict of interest to declare for this manuscript.

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