Elevated Plasma Retinol-Binding Protein-4 are Related with Glucose Dysregulation in Type 2 Diabetes Mellitus

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One of the important adipocytokines, retinol binding protein 4 (RBP4) is thought to play a key role in the pathogenesis of insulin resistance type 2 diabetes mellitus (T2DM) and obesity. This raises the possibility that RBP4 levels could be used for assessing risk of T2DM and further that RBP4 may play a causal role in insulin resistance. But the causal association of the adipocytokine with the basic defects of T2DM (insulin secretory defect and insulin resistance) are still not settled. Therefore, the present study has been designed to investigate the association of RBP4 with insulin resistance in diabetic subjects. A total of 41 T2DM subjects were recruited purposively and 51 healthy subjects served as control. Anthropometric measures and total body fat mass were determined. Glucose, lipids, insulin, RBP4, insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were estimated in these subjects. The study subjects in the 2 groups were age- and BMI- matched. Waist to hip ratio and total body fat mass among the groups did not show statistical difference between groups. Fasting triglyceride and cholesterol were significantly higher in type 2 DM the groups compared to controls (p=<0.001 and 0.005 respectively). Mean HDL did not show significant difference between groups. Fasting insulin (µU/ml) in the T2DM groups was significantly higher compared to the controls (p=<0.001). HOMA%B was 110.0±44.6 and 78.9±17 in control and T2DM respectively; the value was significantly higher in T2DM (p=0.019) compared to the control. HOMA%S was 68.1±17.6 and 56.9±17.1 in Control, was significantly higher in the T2DM groups compared to controls (p<0.001). Mean (±SD) fasting plasma RBP4 in Control and T2DM groups was 30.4±6.0 and 37.2±6.8 respectively. Mean RBP4 value in T2DM was significantly higher compared to the control group (p<0.001). Bivariate Pearson's correlation analyses were then performed for plasma RBP4 with anthropometric and biochemical variables. In control group plasma RBP4 showed significant positive correlation with serum triglyceride (p=0.001) and subscapular-tricep skin fold ratio (p=0.031), but did not showed any association with other testing variables. Plasma RBP4 did not show any association with other testing variables in T2DM group. Multiple linear regression analyses were then performed with HOMA%B as dependant variable and fasting glucose, BMI and triglyceride as independent variables, HOMA%B as dependable variable showed significant negative association with fasting glucose in all four models. In regression analyses with HOMA%S as dependent variable and fasting glucose, BMI and TG as independent variables showed association of HOMA%S with fasting glucose and TG. Multiple linear regression analyses were then performed with RBP4 as dependant variable and fasting glucose, BMI, waist circumference and triglyceride as independent variables. RBP4 showed no association with the variables. RBP4 are not associated with glucose intolerance in mild to moderately overweight T2DM subjects.

[Dinajpur Med Col J 2013 Jul; 6 (2):189-195]

Key words: Retinol binding protein 4, diabetes mellitus

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Introduction

Diabetes mellitus, long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century.[1] T2DM is characterized by insulin resistance and/or abnormal insulin secretion.[2] The precise pathogenesis and the pathophysiological sequence resulting in insulin resistance still not to be clearly understood. Recent studies on insulin resistant patients focused on the molecular dysregulations which are suggested to play important role in the development of insulin resistance and T2DM.[3] Adipose tissue has traditionally been considered an energy storage organ, but over the last decade, a novel role of the adipose tissue as an endocrine organ has emerged.[4] Adipose tissue is currently known to secrete a large number of factors with diverse functions. These factors include free fatty acids (FFA) with well described physiological and pathophysiological effects on glucose homeostasis,[5] and proteins, termed adipocytokines, that act as autocrine, paracrine, or endocrine fashion to control various metabolic functions. Some of these adipocytokines have been implicated in the development of insulin resistance. However, precise roles of these adipokines have not been clearly understood. Recently characterized adipokine is retinol binding protein 4 (RBP4)[6] have also been implicated in the pathogenesis of type 2 diabetes. However, its role in the glucose dysregulation state still not to be clearly understood. Retinol binding protein (RBP4) which is a single-chain polypeptide glycoprotein, belongs to the lipocalin family.[7] RBP4 has found to be increased in insulin-resistant subjects. Graham et al[6] reported increased serum RBP4 concentration in subjects with obesity or T2DM compared with lean subjects. Insulin resistance was positively associated with serum RBP4 concentration and invoked to be causally related with T2 diabetes. In fact, RBP4 is up regulated in the adipose tissue of several insulin-resistant mouse models.[6] Yang et al (8) demonstrated that RBP4 can impair insulin sensitivity throughout the whole body by modulating glucose homeostasis. However, questions still remain unresolved. Various authors have proposed the possible mechanism of insulin resistance and development of T2DM induced by RBP4.Hence it is important to explore retinoal binding protein 4 (RBP4) in the pathogenesis type 2 diabetes.[6]

Methods

A total of 41 type 2 diabetes mellitus patients were recruited purposively and 51 healthy subjects served as control. Anthropometric measures and total body fat mass were determined. Glucose and lipids were measured by standard biochemical methods. Insulin and RBP4 were estimated by using enzyme linked immunosorbent assay (ELISA). Insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were determined by homeostasis model assessment (HOMA) using HOMA-Sigma software. Data were analyzed by appropriate univariate as well as multivariate tools.

Results

The study subjects in the 2 groups were age- and BMI- matched. Waist to hip ratio and total body fat mass among the groups did not show statistical difference between groups. Fasting triglyceride and cholesterol were significantly higher in type 2 DM the groups compared to controls ($p=<0.001$ and 0.005 respectively). Mean HDL did not show significant difference between groups. Fasting insulin (μU/ml) in the T2DM groups was significantly higher compared to the controls ($p=<0.001$). HOMA%B was 110.0±44.6 and 78.9±17 in Control and T2DM respectively; the value was significantly higher in T2DM

(p=0.019) compared to the control. HOMA%S was 68.1±17.6 and 56.9±17 which was significantly higher in the T2DM groups compared to Controls (p<0.001). Mean (±SD) fasting plasma RBP4 in Control and T2DM groups was 30.4±6.0 and 37.2±6.8 respectively.Mean RBP4 value in T2DM was significantly higher compared to the Control group (p<0.001). Bivariate Pearson's correlation analyses were then performed for plasma RBP4 with anthropometric and biochemical variables. In control group plasma RBP4 showed significant positive correlation with serum triglyceride (r=0.454, p=0.001) and subscapular-tricep skin fold ratio (r=0.305, p=0.031), but did not showed any association with other testing variables. Plasma RBP4 did not show any association with other testing variables in T2DM group. Multiple linear regression analyses were then performed with HOMA%B as dependant variable and fasting glucose, BMI and triglyceride as independent variables, HOMA%B as dependable variable showed significant negative association with fasting glucose in all four models (Table IV). In regression analyses with HOMA%S as dependent variable and fasting glucose, BMI and TG as independent variables showed association of HOMA%S with fasting glucose and TG (Table III).Multiple linear regression analyses were then performed with RBP4 as dependant variable and fasting glucose, BMI, waist circumference and triglyceride as independent variables. RBP4 showed no association with the variables (Table V).

Table I: Clinical characteristics of the study subjects

Parameters	Controls (n=51)	T2DM (n=41)	p Control vs T2DM
Age(years)	41±5	43±6	0.23
BMI (kg/m^2)	24.4±4.1	24.9±3.9	0.53
WHR	0.91±.06	0.93±0.05	0.08
BFM (%)	28.2±6.2	28.0±6.5	0.90

Data were expressed as Mean±SD Unpaired students't' test was performed p<0.05 was level of significance. *n* = number of subjects, BMI=Body mass index, WHR=Waist hip ratio, BFM= Body fat mass.

Table II: Biochemical characteristics of the study subjects

Parameters	Controls (n=51)	T2DM (n=43)	p controls vs T2DM
F Glucose (mmol/l)	5.1±0.41	6.3±0.20	<0.001
2h Glucose (mmol/l)	5.9±1.2	6.3±1.2	<0.001
F insulin (µU/ml)	9.7±2.4	11.3±4.4	<0.001
RBP4 (µg/ml)	30.4± 6.07	32.2± 10.9	<0.001
HOMA% B	107± 25	76.5± 18.0*	0.004
HOMA %S	81.9± 18	72±23	<0.001

Data were expressed as Mean±SD Unpaired students't' test was performed p<0.05 was level of significances. *Significantly different compaired to controls
F Glucose =Fasting glucose; 2h glucose = 2 hours after 75g glucose load glucose; F Insulin= Fasting Insulin; RBP4 = Retinol binding protein: HOMA%B = B cell function assessed by homeostasis model assessment and HOMA%S= Insulin sensitivity assessed by homeostasis model assessmen

Table III: Multiple stepwise regression analysis with HOMA%S as dependent variable and F glucose, RBP4, BMI and TG as confounding variables among control and T2DM subjects

Independent Variables	Model 1		Model 2		Model 3		Model 4	
	β	*P*	*β*	*P*	*β*	*P*	*β*	*P*
F Glucose	-0.506	<0.001	-0.457	<0.001	-0.460	<0.001	-0.376	<0.001
RBP4			-0.228	0.015	-0.215	0.022	-0.144	0.119
BMI					-0.091	0.318	-0.093	0.281
TG							-0.287	0.003
Adjusted R^2	*0.248*		*0.290*		*0.290*		*0.352*	

F glucose, fasting glucose; RBP4, retinol binding protein 4; BMI, body mass index; TG, triglyceride

Table IV: Multiple stepwise regression analysis with HOMA%B as dependent variable and F glucose, RBP4, BMI and TG as confounding variables among control and T2DM subjects

Independent Variables	Model 1		Model 2		Model 3		Model 4	
	β	*P*	*β*	*P*	*β*	*P*	*β*	*P*
F Glucose	-0.697	<0.001	-0.710	<0.001	-0.711	<0.001	-0.714	<0.001
RBP4			0.061	0.441	0.064	0.424	0.062	0.459
BMI					-0.023	0.770	-0.023	0.772
TG							0.009	0.915
Adjusted R^2	*0.480*		*0.478*		*0.472*		*0.466*	

F glucose, fasting glucose; RBP4, retinol binding protein 4; BMI, body mass index; TG, triglyceride

Table V: Multiple stepwise regression analysis of RBP4 as dependent variable with F glucose, BMI, WC and TG as confounding variables among control and T2DM subjects

Independent Variables	Model 1		Model 2		Model 3		Model 4		Model 5	
	β	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*
F Glucose	0.191	0.099	0.184	0.097	0.191	0.082	0.192	0.083	0.167	0.153
BMI					0.171	0.120	0.181	0.156	0.191	0.138
WC							-0.021	0.871	-0.035	0.789
TG									0.088	0.504
Adjusted R^2	*0.023*		*0.105*		*0.123*		*0.111*		*0.104*	

F glucose, fasting glucose; BMI, body mass index; TG, triglyceride; WC, waist circumference

Discussion

The major objective of the present study was to explore the association of plasma RBP 4 with the basic pathophysiology of diabetes, namely insulin secretory defect and insulin resistance, in diabetic subjects. Both healthy and Type 2 DM controls were used to compare the results. The age and BMI of the two groups were matched (Table I) which excluded the possibility of the interference by two of the most important confounders of adipocytokines. Again the association between plasma RBP4 and T2DM has previously been shown in only obese (BMI, 31.6±4.5) subjects.[6] Moreover, Kloting et al[10] observed association of RBP4 with obese IGT (BMI, 35.0±6.3). But, in present study an association has been demonstrated even in predominantly mild to moderate overweight T2DM (BMI, 24.9±3.9) subjects. Although the adipokine was raised in the hyperglycemic states, there was no significant correlation of fasting blood glucose with RBP4 in univariate as well as multivariate analysis when adjusted with BMI, waist circumference and TG. The present findings indicate that blood glucose itself may not be a major determinant of circulating RBP4.

The association of the RBP4 with insulin secretory capacity and insulin sensitivity were analysed with univariate as well as multivariate analyses RBP4 was not associated with insulin secretory function as well as insulin sensitivity when adjusted with BMI and TG (Table IV and V) in T2DM subjects. Broch et al[11] found a negative correlation of B-cell seretory capacity with RBP4 in T2DM subjects

Conclusion

From the above data it can be conluded that T2DM have both insulin resistance and secretory defects, and RBP4 are not associated with glucose in mild to moderately overweight T2DM subjects.

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