Is serum retinol binding protein-4: A predictor for diabetes in genetically high risk population?

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Background: Retinol binding protein-4 (BP-4) a new adipocytokine, specifically binds to retinol, through experimental studies, reported its link between obesity and insulin resistance (IR). But till date no studies are available on influence of genetic predisposition of diabetes on RBP-4 expression. Hence, we aimed to study the influence of genetic predisposition of diabetes on the serum RBP-4 and its role in development of IR and diabetes in genetically high risk population. **Materials and Methods:** Healthy non diabetic individuals (age 18 to 22) were grouped into Group I: Control (n = 81), whose parents are non diabetic, non hypertensive and does not have any family history of coronary heart diseases. Group II: (n = 157) with one of their parents diabetic and Group III: (n = 47) with both parents diabetic. In all the participants, we estimated fasting serum RBP-4, insulin and glucose. Homeostasis model for assessment-insulin resistance (HOMA-IR) and homeostasis model for assessment-beta cell dysfunction (HOMA-B) were calculated from fasting serum insulin and glucose levels. **Results:** In this study, we observed significantly higher RBP-4 levels 12.71 ± 2.3 in Group-II and 13.25 ± 2 in Group-III, respectively when compared to Group-I 11.4 ± 1.8 (P < 0.01). RBP-4 showed a significantly strong positive correlation with plasma insulin, glucose and HOMA-IR in genetically high risk population (group II and III) P < 0.01. Linear regression analysis revealed a strong positive association of RBP-4 with parental diabetes even after adjusting for BMI, age and sex (OR 1.53, 95% CI 1.089-1.40). **Conclusion:** Higher serum RBP-4 and its positive correlation with Insulin, glucose, and HOMA-IR in healthy non diabetic participants of genetically high risk population, indicating its role as predictor for the onset of diabetes in coming future.

Key words: Adipocytokines, diabetes, insulin resistance, retinol binding protein-4

INTRODUCTION

In recent times, the status of adipose tissue has changed from mere source of energy store to endocrine organ on discovery of many adipokines and their role in energy homeostasis.[1] RBP-4 a new adipose tissue derived cytokine which specifically binds to retinol, has recently been reported to prove a link between obesity and insulin resistance. It was first found to be expressed in rodent adipocytes in 1992 by Tsutsumi C et al.[2] Later RBP-4 was identified as an adipocytokine that is increased in circulation in mouse models of obesity and insulin resistance.[3] In the experimental study done by Yong Q et al.[4] they observed over expression of RBP-4 in adipose tissue of adipocyte specific GLUT4 knockout mouse.

Furthermore, they observed development of insulin resistance in mice that were either over expressing RBP-4 or were injected with recombinant RBP-4, where as RBP-4 knockout mice showed increased insulin sensitivity. Treatment of GLUT4 knockout mice with TZD resulted in decreased RBP-4 expression, though there was no change in RBP-4 expression in control mice. In addition to animal studies, few human studies done by[5-7] showed positive correlation of RBP-4 with insulin resistance and diabetes. Very few studies like study done by Qibin Qi et al.[8] are available on RBP-4 levels in different ethnic population, but no studies are available on the influence of genetic predisposition of diabetes on RBP-4 expression. As per international diabetic federation India is so called as diabetic capital of the world, with already existing 50.8 million diabetic patients and expected to raise up to 87 million by 2030,[9] not even single study is done till date from Indian subcontinent on the influence of RBP-4 in diabetes. Hence in this study, we investigated the RBP-4 expression and its role in development of insulin resistance and diabetes in genetically high risk population, so that a new pharmacological, diagnostic and prognostic approach can be made in treating diabetes.

MATERIALS AND METHODS

This cross sectional study was done in 2011 at L. N. Medical college-Bhopal, INDIA and is approved by institutional ethics committee with wide Ref No: IEC/3/2011 and written consent was taken from all participants. For this study we selected three groups.

Group I: Control group consists (n = 81) both male and female of age group between 18 to 22 years, irrespective of BMI whose parents are non diabetic, non hypertensive and does not have any family history of coronary heart diseases.

Group II: (n = 157) both male and female of age group between 18 to 22 years, irrespective of BMI with one of their parents with history of type 2 diabetes.

Group III: (n = 47) both male and female of age group between 18 to 22 years, irrespective of BMI, with both parents having history of type 2 diabetes.

Exclusion criteria

None of the subjects from above groups are diabetic, pregnant, gestational diabetic and none of their parents were type 1 diabetic. The diabetes in these subjects was excluded by through applying WHO criteria.[10]

Measurements

All the subjects of above groups are on overnight fasting. In all the above subjects we measured serum glucose by commercial kits for Biosystems A25 fully auto analyzer. Serum insulin and RBP-4 were estimated by ELISA method with commercial kits.

Insulin resistance and β cell dysfunction were measured by homeostasis model for assessment (HOMA) based on formula:[11]

HOMA-IR = fasting serum insulin (μIU/ml) × fasting serum glucose (mg/dl)/405

HOMA-B = fasting serum insulin (μIU/ml) × 3.33/(fasting serum glucose (mg/dl)-3.5).

Statistical analysis

ANOVA is applied to see the statistical difference in the mean values of serum biological parameters, BMI, HOMA-IR and HOMA-B between three groups. Simple student *t*-test was applied to see the difference in the mean values of RBP-4 and other diabetic markers between two groups. Relationship between continuous variables was expressed by applying Pearson's correlation (r) for normally distributed variables and Spearman's correlation for non parametric distribution. Descriptive results of continuous variables are expressed as mean ± SD for normally distributed or as median for nonparametrically distributed variables. P value <0.05 is considered significant and <0.01 as highly significant. Multivariate logistic regression was performed in stepwise manner to evaluate the association among independent and dependent variables. All the data were analyzed using statistical software SPSS version-16 Chicago USA.

RESULTS

Serum RBP-4 and other biological parameters in different study groups

As shown in Table 1, serum RBP-4 levels are significantly high in genetically high risk population with 13.25 ± 2 in both parents diabetic group and 12.71± in single parent diabetic group when compared to control group whose RBP-4 levels are 11.44 ± 1.8 ($P < 0.001$). The same variation in other known diabetic markers like fasting glucose, insulin, HOAM-IR and BMI were observed between study groups and control ($P < 0.01$).

Serum RBP-4 and other biological parameters between single and both parents diabetic population

Table 2 shows statistically high significant difference in serum RBP-4, Insulin, glucose and HOMA-IR between these two groups with highest levels in children of both diabetic parents group ($P < 0.01$). But at the same time no statistical difference in mean BMI and HOMA-B levels between these two groups ($P > 0.05$).

Gender difference in serum biological parameters

In this study, we observed no mean difference in serum biological parameters like insulin in males 10.17 ± 2 and in females 10.36 ± 1.6 ($P > 0.05$), glucose in males 77.44 ± 44 and 77.39 ± 10.2 in females ($P > 0.05$), RBP-4 in males 12.28 ± 3.3 and 11.98±.27 in females ($P > 0.05$), BMI in males 22.67 ± 3.7 and 22.29 ± 3.9 in females ($P > 0.05$). The same no variation in HOMA-IR and B between male and female participants ($P > 0.05$) was observed.

Correlation and association of different diabetic markers with RBP-4 in genetically high risk population

In examining the relationship between serum RBP-4 and other biological markers for diabetes in genetically high risk population before the onset of diabetes, we observed highest significant positive correlation of BMI with RBP-4 (Pearson coefficient 0.844) followed by HOMA-IR (Pearson coefficient 0.47) and fasting glucose (Pearson coefficient 0.439). No statistically significant correlation was observed between RBP-4 and HOMA-B with Pearson coefficient 0.002 ($P > 0.5$).

In assessing the association of RBP-4 and other diabetic markers in study population, Table 3 showing the logistic
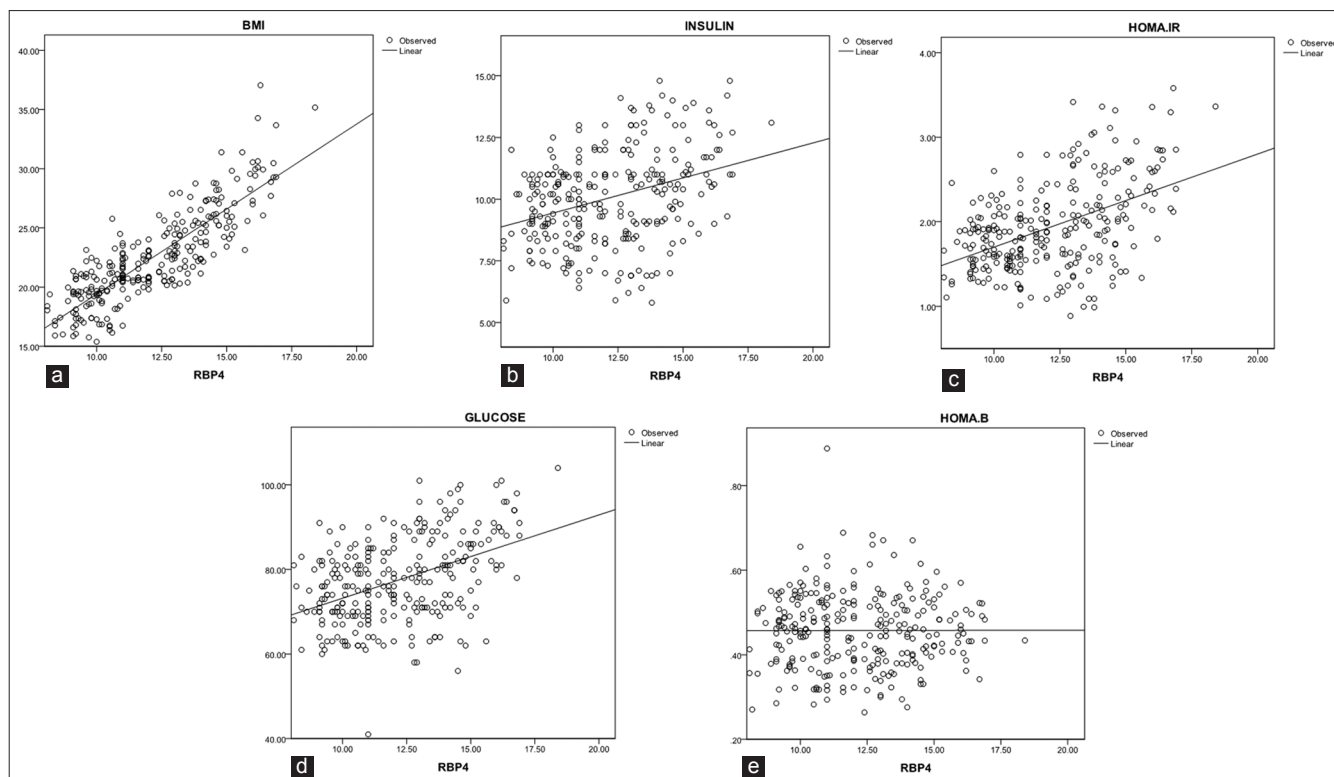
Figure 1: Relationship between RBP-4 and diabetogenic factors

Table 1: One-way ANOVA (analysis of variance) of biological parameters between three study groups

Parameters	Non diabetic (*n*=81)	Single parent diabetic (*n*=157)	Both parents diabetic (*n*=47)	*P* value
BMI (kg/m^2)	21.8±3.3	22.4±4.1	23.6±3.5	0.042*
Fasting serum insulin (µIU/ml)	8.06±0.99	10.79±1.5	11.12±1.3	<0.001**
Fasting serum glucose (mg/dl)	73.4±8.5	78.02±10.1	82.4±9.7	<0.001**
Fasting serum RBP-4 (µg/ml)	11.44±1.8	12.71±2.3	13.25±2.0	<0.001**
HOMA-IR	1.45±0.23	2.09±0.49	2.26±0.41	<0.001**
HOMA-B	0.39±0.07	0.48±0.07	0.47±0.07	<0.001**

BMI=Body Mass Index, RBP-4=Retinol binding protein-4, HOMA-IR=Homeostasis model assessment-insulin resistance, HOMA-B=Homeostasis model assessment-beta cell function. Results are presented as mean±SD. **$P<0.01$; *$P<0.05$

Table 2: Mean difference in serum RBP-4 and other biomarkers between single parent diabetic and both parents' diabetic groups

Parameters	Single parent diabetic group (*n*=147)	Both parents diabetic group (*n*=47)	*P* value
BMI	22.48±4.1	23.63±3.5	0.086#
Fasting serum insulin	10.79±1.5	11.12±1.3	0.192#
Fasting serum glucose	78.0±10.9	82.44±9.7	0.010**
HOMA-IR	2.09±0.49	2.26±0.41	0.030*
HOMA-B	0.48±0.07	0.47±0.07	0.339#
Fasting serum RBP-4	12.17±2.3	13.25±0.07	0.006**

BMI=Body Mass Index, RBP-4=Retinol binding protein-4, HOMA-IR=Homeostasis model assessment-insulin resistance, HOMA-B=Homeostasis model assessment-beta cell function. Results are presented as mean±SD. **$P<0.01$; *$P<0.05$; #$P>0.5$

Table 3: Multivariate logistic regression in stepwise manner to assess the association of RBP-4 with parental diabetes

Parameter	Odds ratio (or)	95% confidence interval CI	*P* value
BRP-4	1.23	1.08–1.40	0.001**
Unadjusted	1.49	1.18–1.87	0.001**
Adjusted for BMI	1.24	1.09–1.41	0.001**
Adjusted for age	1.26	1.11–1.44	0.000**
Adjusted for sex			

BMI=Body Mass Index, RBP-4=Retinol binding protein-4. **$P<0.01$

regression in stepwise manner with parental diabetes as dependent variable, we observed a significant positive association of RBP-4 with the risk of developing diabetes in genetically high risk population with OR 1.235 and *P* 001.

DISCUSSION

In the present study, we observed that serum RBP-4 levels were elevated in study groups of genetically high risk population ($P<0.01$) when compared to study group from low risk population indicating its potential role in initiating and propagating diabetes, metabolic syndrome and coronary heart

diseases in genetically predisposed population. Young Min Cho *et al.*[6] in their study on RBP-4 levels in type 2 diabetes and impaired glucose tolerance also observed very higher serum RBP-4 (18.9 ± 11.2). As expecting there is a gross variation in serum fasting glucose, insulin and levels of HOMA-IR in genetically predisposed healthy individuals when compared to their counterparts whose parents are non diabetic.

At the same time this study showed statistically significant difference in serum RBP-4 with highest levels in individuals whose both parents are diabetic than that of individuals of single parent diabetic ($P < 0.01$), suggesting the genetic variation in the expression of RBP-4. There might be some metabolites expressing either abnormally or over expressed in genetically predisposed population, who might influencing the levels of RBP-4 and its adverse role in developing diabetes.

In this study from Figure 1a, we observed positive correlation between BMI and RBP-4 with Pearson coefficient 0.844 in genetically high risk population for diabetes indicating its direct role in initiating metabolic syndrome. In a significant study done by Gragam T E *et al.*,[5] they also observed same positive association with BMI. In a recent study done by Lee DC *et al.*[12] on the association of RBP-4 and insulin resistance in healthy adolescents also observed linear positive correlation between RBP-4 and BMI.

As shown in Figure 1b from our study, we observed that as serum RBP-4 increases serum insulin is also increases predicting its crucial role in creating hyperinsulinemia like environment which prevails in type 2 diabetes. Our hypothesis strongly supported by Kotani K *et al.*[13] study. In their study on treating adip-GLUT-4$^{-/-}$ mice with rosiglitazone, an activator of peroxisome proliferator-activated receptor gamma (PPAR) reversed increased adipocyte RBP-4 mRNA expression and reversing insulin resistance.

From Figure 1c in this study, we observed close correlation between RBP-4 and HOMA-IR depicting its role in developing insulin resistance (IR). It may be through retinol dependent and or retinol independent mechanisms. Retinoids can stimulate phosphoenolpyruvate carboxykinase (PEPCK) expression in liver and hepatic gluconeogenesis.[14] In addition, retinol is a precursor for the synthesis of ligands of retinoic acid receptor and retinoid X receptor. These are the partners of the peroxisome-proliferator-activated receptor family that regulates gene control to fatty acid metabolism.[15,16] RBP-4 could thus be linked to insulin resistance through impaired fatty acid metabolism. Yong Q (3) *et al.* in their experimental study observed increased expression of several retinoid-responsive genes, retinoic acid receptor-2, stearoyl CoA desaturase-1 and acetyl CoA carboxylase in muscle of

RBP-4 transgenic mice, thus increased delivery of retinol by RBP-4 might explain its effect on IR.

The same positive correlation between RBP-4 and glucose was observed in study population whose parents are diabetic [Figure 1d], indicating its glucogenic effect. Yong Q[2] *et al*. also showed that RBP-4 directly stimulates PEPCK expression and glucose production in cell culture, suggesting its direct effect on hyperglycemia. But from Figure 1e we observed no correlation between RBP-4 and HOMA-B indicating no probable role regulation, expression and release of insulin from beta cells.

In contrast to few studies,[17,18] we observed no gender difference in serum RBP-4 levels between male and females. This may be due to the age group which we selected.

CONCLUSION

From this study though we observed no gender difference in RBP-4 expression, statistically significant higher serum RBP-4 and its positive correlation with insulin, glucose, BMI and HOMA-IR in health non diabetic participants of genetically high risk population was observed, signifying its role in initiation and propagation of progressive degenerative diseases like diabetes. At the same time before any significant changes in diabetic markers we observed elevated serum RBP-4 in genetically high risk population indicating its role as predictor for the onset of diabetes in coming future.

ACKNOWLEDGMENTS

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