ORIGINAL ARTICLE

Circulating Levels of Adipokines and TNFα in Patients with and without Type 1 Diabetes

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ABSTRACT

Objective: The aim of this study was to evaluate circulating levels of adiponectin, resistin, visfatin and TNF α in type1 diabetic children and young adolescents.
Patients and Methods:
Design: Cross-sectional study
Setting: Ziauddin University Karachi
Subjects: 90 subjects (30 type I diabetic (T1DM) subjects and 60 non-diabetic controls) between the age of 05-15 years.
Methods: Serum adiponectin, resistin, visfatin, TNF α and insulin were measured using ELISA and fasting blood sugar by glucose oxidase (Standard Kit method).
Results: Out of 90 study subjects, 30 were type 1 diabetic patients and 60 non-diabetic controls. Increased levels of serum adiponectin levels were significant in type I diabetic subjects (TIDM) (10.83+1.73) compared with controls (9.07+1.25). Same pattern was observed for serum TNF α in diabetic subjects (10.82+4.53) compared with controls (7.22+ 2.22). Significantly increased serum visfatin levels were found in TIDM patients (10.89+2.72) compared with controls (4.97+1.57). Significant negative correlation was observed between TNF α and resistin (r=-0.356), adiponectin and age (r= - 0.594), and glucose and insulin in TIDM patients (r= -0.588). Binary logistic shows significant association of HOMA IR, adiponectin and visfatin with Type 1 diabetes mellitus. Level of significance set for the study was P=0.05.
Conclusion: Increased levels of circulating adipocytokines in Type I diabetic children depict the important role of cytokines in Type I diabetic patients. However, increased adiponectin concentration in type I diabetic subjects needs to be further explored in large-scale studies.

Key words: HOMA-IR, Adiponectin, Resistin,Visfatin,TNF α, Insulin.

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INTRODUCTION

Type 1 diabetes, formerly known as juvenile diabetes, causes the body to lose ability to produce insulin, which controls the level of sugar in the blood. Type 1 diabetics are insulin-dependent and the cause may be genetic, environmental or autoimmune. Type 1 Diabetes Mellitus is linked to alleles coded by genes in HLA D region.[1] Report of Wall Street Journal 2012 shows a 23% jump in Type 1 diabetes among American youth, which persuaded the researchers to investigate influence of environmental factors or behavior to the onset of the autoimmune disease.[2] Adipokines secreted by white adipose tissue influence insulin signaling that affects insulin sensitivity. Resistin which is a secretory protein is thought to be involved in insulin resistance, diabetes and inflammation. Study by Shalev et al 2004, showed significantly higher levels of resistin in TIDM patients which decreased to normal after islet transplantation.[3] Contrary to this study by Schaffler et al. 2004 showed lower levels of resistin in TIDM patients compared to controls and no correlation was observed with diabetic complications.[4] Similarly, studies by Akash et al 2012 and Donath and Shoelson, 2011 showed role of inflammatory cytokines in autoimmunity and autoimmune diabetes and administration of TNFα to autoimmune diabetes-prone non-obese mice and rats inhibits diabetes development.[5,6] Similar study by Celi et al , 2006 showed significantly higher levels of adiponectin in prepubertal diabetics compared with

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controls while resistin levels were lower in pubertal diabetics and no difference was observed in TNF α concentrations in Type 1 diabetics and non-diabetic children.[7] Limited and controversial data is available regarding the role of adipokines and TNF α in Type 1 diabetics. Moreover, no such study has been yet published from Asian population. The objective of this study was to determine the circulating levels of adipokines (adiponectin, resistin, visfatin) and TNF α in Type 1 diabetic children and to compare it with nondiabetic controls. Association of adipokines to Type 1 diabetics was also determined.

PATIENTS AND METHODS

Study Design: Cross-sectional Study

Sampling Technique: Convenient sampling

Subjects: Ninety subjects (60 controls and 30 Type 1 diabetics) in age range between 5 to 15 years, who consecutively visited diabetes clinic at Ziauddin University Hospital were recruited in the study.

Study protocol: The consent form was signed either by themselves or by their parents. The study was approved by Ethical Committee, Ziauddin University. Non-diabetic subjects as controls and Type 1 diabetic patients (T1DM) were included. T1DM patients were using short and intermediate acting subcutaneous insulin injections. Individuals having diseases other than the diabetic complications (endocrinological disorders, inflammatory conditions like rheumatoid arthritis, malignancy, etc.) were excluded from the study. Body weight, height, body mass index (BMI) and waist hip circumference was measured by standardized procedures. Estimate of insulin resistance by Homeostasis Model Assessment (HOMA) score was calculated with the following formula:

Fasting serum insulin (µU/ml) x fasting plasma glucose (mmol/l)/22.5. As described by Hedblad et al.,[8] patients with HOMA score values exceeding the 75th percentile (i.e., 2.0) were considered to have insulin resistance Baseline parameters were assessed and inflammatory adipokines, such as adiponectin, resistin, visfatin and tumor necrosis factor were determined. Serum samples for adipokines level were stored at -80^0C until assayed. Fasting plasma glucose was determined by glucose oxidase method. Serum concentrations of insulin adiponectin, resistin and visfatin, TNF α and were determined by quantitative enzyme assays (DRG diagnostics, Germany).[9,10]

Statistical analysis: Statistical Software for Social Sciences "SPSS version-17" was used for statistical analysis. Mean± SD were computed for quantitative variables like age, height, weight, BMI, waist, hip, waist-hip ratio, glucose, insulin, HOMA IR, Adiponectin, Resistin, TNF α, Visfatin.

Pearson Correlation co-efficient was computed (level of significance = 0.05) to determine the association between quantitative variables of two groups i.e. control group and Type 1 diabetic group. .

Student t test was applied to compare the two groups. A value of 0.05 was considered statistically significant. Binary Logistic was applied on Type 1 diabetic group to determine the association of adipokines with TIDM.

RESULTS

Table 1 shows the baseline parameters and serum concentrations of adipokines in TIDM. Serum adiponectin was found to be significantly increased in TIDM type I diabetic subjects (TIDM) (10.83+1.73) compared with controls (9.07+1.25). Serum TNF α showed the same pattern in diabetic subjects (10.82+4.53) compared with controls (7.22+ 2.22). Similarly, serum visfatin was significantly raised in TIDM patients (10.89+2.72) compared with controls (4.97+1.57).

Table 2 shows the correlation of variables with each other in TIDM . Significant negative correlation was observed between TNF α and resistin (r=-0.356), adiponectin and age (r= - 0.594) and glucose and insulin in TIDM patients (r= -0.588). Correlations are also shown in Figure 1.

Table 3 shows Binary logistic highlighting significant association of HOMA IR, adiponectin and visfatin with TIDM .

DISCUSSION

The anthropometric measurements like age, BMI and waist hip ratio of TIDM children were found to be non-significant compared with controls in this study. Adiponectin, visfatin, TNF α and HOMA IR score were significantly increased in type 1 diabetic patients compared with controls, however no significant change was observed in resistin levels amongst the two groups. Adiponectin and visfatin are associated with TIDM. HOMA- IR score correlated significantly with age ($P<0.05$) and insulin negatively with glucose (r = -0.588) in T1DM children. On the contrary, reduced levels of adiponectin were observed in type 2 diabetic patients as reported by several studies.[11,12] However,

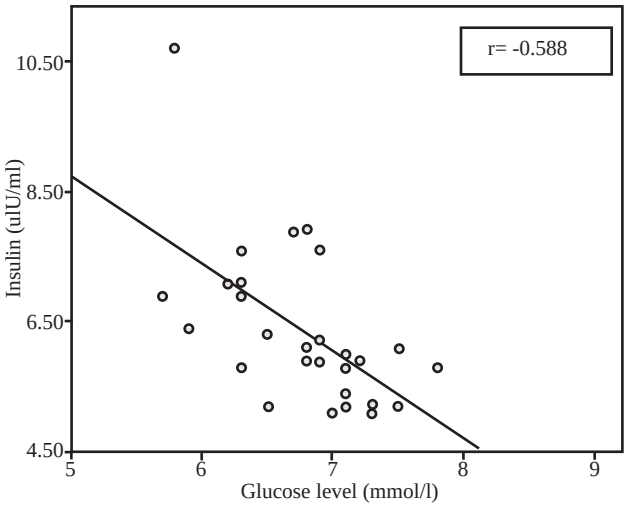
Table 1: Baseline characteristics of controls and diabetic Type 1 subjects

The values are expressed as mean and standard deviation. The number of subjects is given in parenthesis.

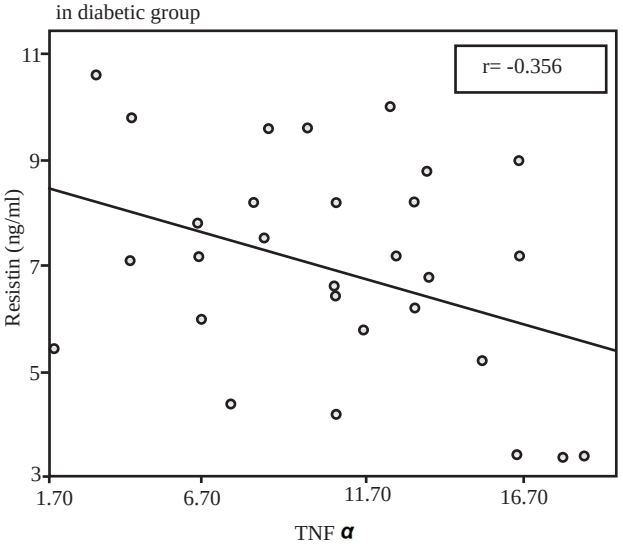
Variables	Control Group (n=60) (Mean ±SD)	Type I diabetic Group (n=30) (Mean ±SD)
Age (yrs)	9.13 ± 2.02	8.23 ± 2.78
Height (m)	1.18 ± 0.06	1.16 ± 0.65*
Weight (kg)	22.93 ±2.47	23.16 ± 2.27*
BMI (Kg/m^2)	16.23 ± 1.236	17.09 ± 1.78*
Waist (cm)	55.45 ± 4.73	56.27 ± 4.65*
Hip (cm)	63.55 ± 5.52	65.87 ± 6.04*
Waist - Hip Ratio	0.86 ± 0.05	0.85 ± 0.05*
Glucose level (mmol/l)	5.05 ± 0.58	6.78 ± 0.52*
Insulin (uIU/ml)	7.07 ± 0.99	6.35 ± 1.17*
HOMA IR	1.60 ± 0.34	1.79 ± 0.17*
Adiponectin (ng/ml)	9.07 ± 1.25	10.83 ± 1.73*
Resistin (ng/ml)	6.57 ± 2.47	6.91 ± 2.09
TNFα (ng/ml)	7.22 ± 2.22	10.82 ± 4.53*
Visfatin (ng/ml)	4.97±1.57	8.50± 2.14*

*P<0.05 -Significant compared with Controls

Figure 1: Correlation between Glucose Level & Insulin (ulU/ml) in Type I Diabetic Group



Correlation between Resistin (ng/ml) and TNFα (ng/ml) in diabetic group



significant increased concentration of adiponectin in T1DM children in our study is consistent with the studies done by Imagawa et al 2002 and Perseghin et al 2003,[13,14] who reported increased concentration in adults. One of the possible causes may be the lesser effect on development of insulin sensitivity in children especially at prepubertal stage.[15] Several other reasons for higher adiponectin levels in type 1 diabetic patients are discussed in literature according to which insulin resistance leads to increased concentrations of insulin in Type 2 diabetics which in turn decreases adiponectin concentration, conversely lack of endogenous insulin in type 1 diabetes leading to increased adiponectin concentrations.[16,17] Moreover (subclinical) diabetic complications like cardiac disease or renal dysfunction may push up adiponectin levels even further.[17,18] Further research is needed to evaluate adiponectin-stimulating pathways and to determine whether elevated adiponectin levels in such situations are functioning to attenuate vascular stress or otherwise.[19,20] Study by Jaber al Ahmed (2012) has shown that the levels of adiponectin remain higher in persons with type 1 diabetes than in non-diabetics, even after tackling obesity and HDL cholesterol.[16]

Resistin levels were not found to be significantly increased in T1DM children compared with non-diabetic controls nor did they show any correlation with the BMI, fasting glucose, insulin or HOMA IR score which is consistent with the findings of Celi et al 2006.[7] Study on T1DM in adults shows contrasting results as reported by Shalev et al 2004 and Fehmann et al 2002.[1,21]

This study shows increased concentrations of TNF α and visfatin in TIDM children compared with non-diabetics. However, the study by Cele et al 2006[5] shows no significant difference in concentrations of TNF α in both of the groups. Goldberg et al 2009 showed increased concentration of TNF α in TIDM which is consistent with our study.[22] Toruner et al 2009[23] reported lower visfatin concentrations in TIDM patients than

controls, however Stadler et al 2009, indicated increased concentrations of visfatin in TIDM patients than controls.[24] As limited studies are done on the role of TNF α and visfatin in TIDM, more studies are needed to confirm it.

CONCLUSION

Increased levels of circulating adipokines in TIDM children highlight the important role of adipokines in TIDM patients. However, increased adiponectin concentrations in type I diabetic subjects need to be further explored in large-scale studies.

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Table 2: Correlation of Variables in Type 1 diabetic group

Variables are expressed as r values

Variables	Age	HOMA Score	Glucose	Insulin	Adiponectin	Resistin	TNF α	Visfatin
Age	-	0.364*	0.054	-0.027	-0.594***	-0.178	-0.52	-0.299
Glucose level (mmol/l)	0.054	-0.036	-	-0.588**	0.270	0.071	0.094	0.133
Insulin (uIU/ml)	-0.027	.0309	-0.588**	-	-0.157	-0.162	-0.230	-0.175
HOMA IR	0.364*	-	-0.36	0.309	-0.231	-0.017	0.121	-0.095
Adiponectin (ng/ml)	-0.594**	-0.231	0.270	-0.157	-	0.122	0.256	0.275
Resistin (ng/ml)	-0.178	-0.017	0.071	-0.162	0.122	-	-0.356	0.113
TNFα	-0.52	0.121	0.094	-0.230	0.256	-0.356	-	0.245
Visfatin (ng/ml)	-0.299	-0.095	0.133	-0.175	0.203	0.113	0.245	

Table 3: Binary logistic (Type I Diabetic group)

Variables	β	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
HOMA IR	4.145	1.802	5.289	1	0.021	63.116	1.845	2158.951
Adiponectin (ng/ml)	0.621	0.284	4.782	1	0.029	1.860	1.067	3.245
Visfatin (ng/ml)	0.012	0.003	12.327	1	0.000	1.012	1.005	1.019
Constant	-21.932	5.492	15.945	1	0.000	0.000		

The final logistic regression model includes HOMA IR, adiponectin and Visfatin. After adjusting for the effect of other variables in the model and taking the option "Type I diabetic" as the reference category. The model equation is now:

Type I diabetic = -2.1.932 + 0.012(Visfatin) + 0.621(Adiponectin) + 4.145 (HOMA IR)

Nagelkerke R Square shows that 53% to 73% of the variation in the outcome variable (type I diabetic) is explained by this logistic model.

Hosmer Lenshow test for the final model is $\div^2$= 42.39 (p-value<0.05), which shows that the model is not fitted well for the data and model predicts 67% correct data.

The contribution of visfatin (Wald= 12.327) is more than adiponectin and HOMA IR (Wald=4.782 & 5.289) respectively in the model. HOMA IR, adiponectin and visfatin are associated with type I diabetic (p-value of 0.021, 0.029, & <0.05 respectively). The Exp (B) tells the odd ratio. An increase in 1 unit in HOMA IR is associated with 63 folds increases in the odds that a child will be patient of type I diabetic. An increase in 1 unit (ng/ml) in adiponectin is associated with 2 fold increases in the odds that a child will be patient of type I diabetic and an increase in 1 unit (ng/ml) in visfatin is associated with 1 fold increases in the odds that a child will be patients of type I diabetic.

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