ORIGINAL ARTICLE

Status of serum adiponectin related to insulin resistance in prediabetics

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Abstract

Obejctive: To find the status of serum adiponectin in individuals progressing towards Type 2 diabetes mellitus and compare it with normal glucose tolerant subjects to determine the stage where alteration of adiponectin occurred.

Methods: The cross-sectional study was carried out at the Department of Biochemistry, Jinnah Postgraduate Medical Centre, Karachi, during January to August 2008. Subjects were invited through various diabetes screening camps. A total of 608 subjects ≥30 years of age without prior history of diabetes were screened through fasting plasma glucose and 2-hour oral glucose tolerance test. Forty randomly selected pre-diabetic subjects and 40 age and gender-matched subjects were included in the study. Anthropometric measurements were done. Serum insulin and adiponectin were estimated by enzyme-linked immunosorbent assay. Homeostasis model assessment of insulin resistance (HOMA-IR) was used to calculate insulin resistance mathematically.

Result: Mean fasting and two-hour plasma glucose, body mass index, waist, hip circumference and blood pressure were significantly raised in pre-diabetics compared to those with normal glucose tolerance. Adiponectin was significantly decreased, while insulin and HOMA-IR were raised significantly in the pre-diabetics. Adiponectin showed significant negative correlation with body mass index ($r=-0.31$, $p=0.005$), fasting plasma glucose ($r=-0.24$, $p= 0.032$), 2-hour plasma glucose ($r=-0.42$, $p<0.0001$)), insulin ($r= -0.43$, $p<0.0001$) and HOMA-IR ($r= -0.43$, $p<0.0001$) and remained significant after adjustment of body mass index, gender and insulin level in pre-diabetics.

Conclusion: Adiponectin estimation may help in earlier identification of impending diabetes. However, casual link between adiponectin and pre-diabetes remained unexplored due to the study design and small sample size that warrants longitudinal large-scale studies.

Keywords: Adiponectin, Pre-diabetes, Insulin resistance. (JPMA 64: 184; 2014)

Introduction

The prevalence of diabetes and its adverse effects are rising very rapidly all over the world, significantly adding to the burden of preventable diseases.[1] In Pakistan, nearly 6.4 million people are suffering from diabetes, while a slightly higher proportion has pre-diabetes (7.2million) that may be expected to rise more rapidly.[1,2] The International Diabetic Federation (IDF) predicted that by 2030, Pakistan will have approximately 11.4 million adults with diabetes, the fourth largest population after India, China and the USA.[1,3]

Development of diabetes is nearly always preceded by the stage of pre-diabetes that includes impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT).[4] Pre-diabetes refers to worsening of insulin resistance.[5] Interventions in this phase prevent or delay progression to diabetes[6] and affect cardiovascular outcomes. Approximately 11% of individuals with pre-diabetes progress to diabetes each year.[5]

Adipose tissue that was viewed as playing the passive role in lipid and energy homeostasis[7] expresses and secretes numerous peptide hormones, collectively called adipocytokines.[8] Adiponectin is the only adipocytokine that has insulin-sensitising property.[9] Decline in adiponectin was observed prior to hyperglycaemia and glucose intolerance in Rhesus monkeys that improved when adiponectin was administered.[10] It restored insulin sensitivity in insulin resistant mice.[11]

Prospective and longitudinal studies indicated that lower adiponectin level was associated with higher incidence of insulin resistance and type 2 diabetes mellitus (T2DM) in humans.[12-14] These experimental studies and epidemiological observations that lower adiponectin levels reflect greater diabetes risk suggest the hypothesis that alteration in adiponectin play part in progression from normal glucose tolerance (NGT) to pre-diabetes. This study carried an important implication as understanding the hormonal status of pre-diabetes may add another factor beyond the currently known risk factors for pre-diabetes. We attempted to find the status of serum adiponectin in pre-diabetics and compared it with NGT subjects to determine the stage where and when

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alteration of this hormone had occurred.

Patients and Methods

The cross-sectional study was carried out at the department of Biochemistry, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, from January to August 2008. Subjects were recruited through various diabetes screening camps. The sampling procedure was in line with literature[15] (Box). The study participants were reassured about the confidentiality of data and an informed consent was signed by each participant. The study was approved by the institutional ethics committee.

Due to unpredictability in finding the pre-diabetics, the priori sample size for the present study was not calculated for analysing the correlation. Screening for pre-diabetes was thus continued during the study period. However, a post hoc calculation was done to estimate the power of the study by G-power 3.1.6 software[16] which showed a power of 79%, with a two-sided α-value of 0.05. This demonstrates sufficient power to detect clinically meaningful correlations between the analysed groups.

A total of 608 subjects ≥30 years of age were subjected to fasting plasma glucose (FPG) and two-hour oral glucose tolerance test (OGTT). A sampling frame of all the participants attending the screening camps was constructed and subsequently categorised on the basis of their FPG and two-hour OGTT values into normal, pre-diabetic and diabetic groups. Participants with plasma glucose values above normal, but below diabetic range (IFG±IGT) constituted pre-diabetic group. From the sampling frame, only 40 pre-diabetic subjects were randomly selected. Age and gender matched subjects from the NGT group were selected as control. Serum insulin and adiponectin levels of only these selected NGT and pre-diabetic subjects were estimated from the samples drawn in the fasting state i.e before giving 75gm of glucose.

Box: Procedure of Sample Collection.

Diabetes screening camps organized
↓
Known diabetics, individuals with endocrine disorder or taking any medication that alter blood glucose level were excluded
↓
Participants fulfilling the inclusion criteria invited to participate (Either sex, of age ≥30 years, no history of diabetes)
↓
Participants instructed to maintain overnight fast of at least of 10-12 hour
↓
Next day in the morning questionnaire administered, data collected and anthropometry done (n= 608)
↓
3-4 ml blood drawn in two containers, samples transported with in one hour to the lab for estimation of fasting plasma glucose and separation and storage of serum
↓
Serum stored at -70 0 C for later estimation of Insulin and Adiponectin
↓
75 g glucose dissolved in 250 ml of water given orally
↓
Two hours after glucose ingestion, 3 ml blood collected for estimation of plasma glucose only

Plasma glucose (FPG and 2-h postload) was measured by glucose oxidase-peroxidase method using kit no GL1012 manufactured by Randox UK using a Hitachi-912 Autoanalyzer (Hitachi, Mannheim, Germany). Serum insulin and serum adiponectin were determined by monobind insulin microplate enzyme-linked immunosorbent assay (ELISA) kit no 2425-300 manufactured by Monobind Inc, USA and Biosource adiponectin ELISA kit reference no. KAPME 09 manufactured by Biosource diagnosis Belgium respectively.

Statistical comparisons between categorical variables were made by chi-square test and comparison between continuous variables were made by independent sample 't' test. Pearson's correlation coefficient (r) was used to establish the association of serum adiponectin with body mass index (BMI), FPG, 2-hour PG, insulin and homeostasis model assessment of insulin resistance (HOMA-IR). Regression analysis was done to determine the effect of BMI, gender and insulin on serum adiponectin level. All p-values presented were two-tailed. The statistical tests were considered significant at the level < 5%. All statistical analyses were performed using SPSS 13.0 software.

Results

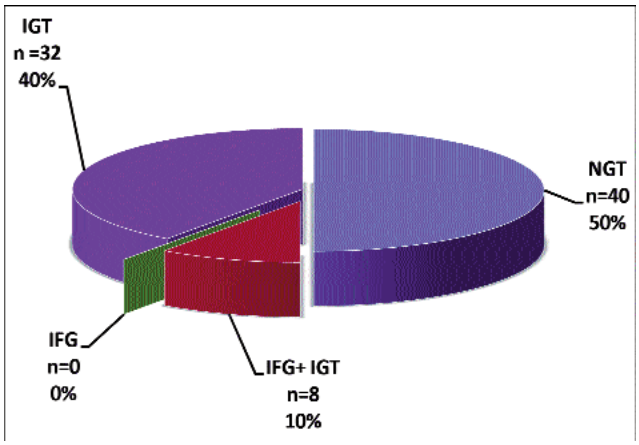
Forty pre-diabetes subjects were selected regardless of gender on the basis of FPG and two-hour OGTT in addition to age and gender-matched NGT subjects for comparison. Gender-wise distribution of the study participants in control and pre-diabetes groups showed non-significant difference ($X2 = 0.464$, $p > 0.05$).

Of the total 80 subjects in the study, 8 (10%) had both IFG and IGT (FPG= ≥110-125 mg/dl; and 2-hour OGTT ≥140-200mg/dl). Besides, 32 (40%) participants had isolated IGT, (FPG ≤110mg/dl; but 2-hour OGTT ≥140-≤200 mg/dl). The remaining 40 (50%) subjects were NGT (FPG ≤110mg/dl; and 2-hour OGTT ≤140mg/dl) (Figure).

Table-1: Comparison of demographic, anthropometric, clinical and biochemical parameters of Normal Glucose tolerance and Pre-diabetes.

S.No	Biophysical Parameters	Normal Glucose Tolerant (Control)	Pre-diabetes
1	Age (Years) n=40	38±0.8 (30- 49)	39±0.9 (30-51)
	Male (n = 25/22)	39 ± 1.2	42 ± 1.3
	Female (n = 15/18)	35 ± 0.6	35 ± 0.7
2	BMI (kg/m2)	22.5±0.41 (20.69-32.45)	25.1±0.40** (23.84-37.31)
	Male	22.1 ± 0.53	25.2** ±0.58
	Female	23.3 ± 0.59	24.8 ± 0.54
3	Waist (cm)	86±1.3 (69-101)	94±1.2** (78-109)
	Male	88 ± 1.6	96** ± 1.7
	Female	84 ± 2.1	91 ± 1.3
4	Systolic B.P (mm Hg)	117±1.8 (90-150)	124±3.0* (100-210)
	Male	118 ± 1.7	125* ± 4.9
	Female	115 ± 2.6	122 ± 3.1
5	Diastolic B.P (mmHg)	80±1.3 (70-110)	86±1.8* (70-130)
	Male	83 ± 1.7	91* ± 2.4
	Female	77 ± 1.6	80 ± 1.7
6	Insulin (µIU/ml)	11±0.53 (6.4-19.3)	16.8±0.89** (4.3-30.4)
	Male	10.4 ± 0.64	17.2**± 1.33
	Female	12.1 ± 0.86	16.4**± 1.17
7	Adiponectin (ng/ml)	11.4±0.78 (5.2 - 23.8)	8.2±0.52** (1.81 - 14.7)
	Male	9.6 ± 0.74	7.7 * ± 0.58
	Female	14.6 ± 1.35	8.9**± 0.91
8	HOMA-IR	2.48±0.14 (1.06-4.85)	4.13±0.24** (1.32-8.18)
	Male	2.31 ± 0.16	4.31 ± 0.36
	Female	2.77 ± 0.26	3.92 ± 0.31

Student t-test used for comparison. Values expressed in mean±s.e.m (range). * p<0.05, * * p<0.01.



NGT= Normal Glucose Tolerant. Fasting Plasma Glucose (FPG)= <110 mg/dl and 2-hour Oral Glucose Tolerance Test (OGTT) <140 mg/dl.
Pre-diabetes includes at least any one of following:
IFG= Impaired Fasting Glucose (FPG= ≥110-<126 mg/dl)
IGT= 2-hour Impaired Glucose Tolerance (2-hour OGTT ≥140 - <200 mg/dl)
IFG+IGT= Both Impaired Fasting Glucose and 2-hour Impaired Glucose Tolerance.

Figure-1: Distribution of study participants into Normal Glucose Tolerant and Pre-diabetes on the basis of fasting and 2-hour plasma glucose values.

Table-2: Pearson's correlation of adiponectin with fasting plasma glucose, 2-hour plasma glucose, BMI, insulin and HOMA-IR.

S.No		Adiponectin r (p)
1	FPG	- 0.24 (0.032)
2	2-hour plasma glucose	- 0.42 (0.001)
3	BMI	- 0.31 (0.005)
3	Insulin	- 0.43 (0.001)
4	HOMA-IR	- 0.43 (0.001)

Pearson's Correlation (r) of fasting plasma adiponectin with fasting plasma glucose, 2 hour plasma glucose, BMI, insulin and HOMA-IR. p values shown in parenthesis. FPG: Fasting Plasma Glucose; BMI: Body Mass Index.

Table-3: Stepwise multivariate linear regression analysis with serum adiponectin as dependent variable.

Model		Standardised Coefficients	P value
		Beta	
I	BMI	-0.308	0.005
II	BMI	-0.347	0.001
	Gender	0.349	0.001
III	BMI	-0.177	0.099
	Gender	0.361	0.000
	Insulin	-0.379	0.001

A 'p' value of <0.05, considered statistically significant.
BMI: Body Mass Index.

Comparison of demographic, anthropometric, clinical and biochemical parameters of the NGT subjects with prediabetes were done (Table-1). Significant difference was observed in mean waist circumference, systolic blood pressure, diastolic blood pressure, insulin, adiponectin and HOMA-IR values between the two gruops. Adiponectin showed significant negative correlation with FPG, 2-hour plasma glucose, BMI, insulin and HOMA-IR (Table-2). To determine the effect of independent predictor's variables on the dependent variable serum adiponectin level, a multivariate stepwise linear regression analysis was done (Table-3). Analysis by multivariate regression analysis showed adiponectin remained significant after adjustment for BMI, gender and insulin level.

Discussions

The current study revealed several important findings. First it confirmed previous findings about gender disparity in adiponectin level, with women having higher mean adiponectin than men.[17,18] This disparity in hormonal status was maintained even in pre-diabetic group. Gender dimorphism in hormonal level may be due to regional adipose tissue distribution. Men have greater propensity to accumulate excess fat within the

abdominal cavity (visceral fats).[19] With accumulation of visceral fat, adiponectin level was found to be decreased. This decline in adiponectin could be due to decreased adiponectin messenger ribonucleic acid (mRNA) level in visceral fats compared to subcutaneous fats.[20] Halleux et al[21] hypothesised that there are some factors that destabilize adiponectin mRNA in visceral adipose tissue. Therefore difference in visceral and subcutaneous adipose tissue accumulation between both genders is an important factor in explaining the gender differences in adiponectin level.

With non-significant difference in age, pre-diabetic groups in the current study had significantly increased mean waist circumference, BMI, and systolic and diastolic blood pressure. Increased waist circumference - a strong risk factor for the progression towards diabetes - is the result of increased visceral adipose tissue accumulation.[22] Studies pronounced that people of South Asian descend have higher incidence of obesity-related complications than other ethnic groups due to increased abdominal fat distribution.[23,24] Abdominal fat deposition is markedly high for a given level of BMI in the Asians than in the Europeans. Asians, therefore, have increased predisposition to diabetes which is not explained by conventional risk factors. Thus, identification of new and novel risk markers released from the fat cells like adiponectin could facilitate the early detection and may thereby lead to risk-reduction of diabetes.

Significantly raised BMI in pre-diabetics suggested that progression in obesity and glucose dysregulation runs parallel to each other. The pre-diabetics in the current study were overweight as per BMI cutoff for European populations, but were obese when revised BMI criterion for Asian population was applied. Presence of glucose dysregulation at this mean BMI value reinforces the use of revised criteria for defining the obesity in Asian population.[25]

Another important observation of the current study is the finding of strong inverse association of fasting insulin and insulin resistance with serum adiponectin level. A strong correlation between adiponectin and systemic insulin sensitivity has been established both in vivo and in vitro, in animal as well as in human studies.[10-14] In a longitudinal study, circulating adiponectin levels has been shown to decrease in parallel with progression of insulin resistance during development of T2DM in Rhesus monkeys. These monkeys are genetically predisposed to develop insulin resistance. In these monkeys, decline in adiponectin levels preceded overt hyperglycaemia.[26]

The current study extends the above finding by exploring that the plasma adiponectin concentration is more related to insulin resistance and fasting insulinaemia in subjects with pre-diabetes than to glycaemia, which suggests that hypoadiponectinaemia in people with pre-diabetes is largely attributable to insulin resistance and/or hyperinsulinaemia. But, is hyperinsulinaemia per se a mediator of low adiponectin levels? The answer to this question can be best explained by a longitudinal study. The current study had all the inherent limitations of cross-sectional design, and that warrants a careful interpretation of the findings. However, finding of significantly lower adiponectin level even after adjustment of BMI, gender and insulin level indicated that decline in adiponectin is an independent phenomenon not related to BMI, gender and insulin level in pre-diabetic subjects. A similar observation was reported by Chandran et al who found lower adiponectin in the later stages of T2DM when circulating insulin levels had declined.[27]

Conclusion

The study supports the assumption that adiponectin may play an important role in the pathogenesis of abnormal glucose metabolism. However, further investigations are needed to elucidate the causal relationship that warrants a longitudinal large-scale study.

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