OCTOBER-DECEMBER 2012

MEDICAL CHANNEL

Original Article

ROLE OF ADIPONECTIN AS A BIOMARKER OF PREDIABETES

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ABSTRACT:

Background: Prediabetes is aninsulin resistant state. It includes subjects with impaired fasting glucose and/or impaired glucose tolerance.Adiponectin is a peptide hormones synthesize exclusively from adipose tissues and has insulin sensitizing property.Decreased adiponectin was observedin diabetes and in insulin resistant states in various studies. Adiponectin has been proposed as a biomarker of metabolic syndrome.
Aim: We attempted to find out the role of serum adiponectinas a biomarker of prediabetes.
Material & Methods: This cross sectional study was carried out in the Department of Biochemistry, BMSI, JPMC, Karachi during Jan to Aug 2008. Subjects were invited through various diabetes screening camps. A total of 608 subjects e" 30 years of age with out prior history of diabetes were screened through FPG and 2 hour OGTT. Individuals with diabetes were excluded from the study.
Forty randomly selected prediabetic subjects and forty age and sex matched NGT subjects were included in the study. Anthropometric measurements were done. Serum insulin and adiponectin were estimated by ELISA. HOMA-IR was used to calculate insulin resistance mathematically.
Mean values ofall the variables of thecontrol subjects weretaken asa reference.Based on their individual values, above or below the mean value, participants of each group was further categorize into two groups. A chi-square test statistics is applied to reject the null hypothesis that adiponectin may notbe used as a biomarker of prediabetes.
Results: For all measured variables, two hour plasma glucose resulted in the best discrimination (X^2 = 21.58, p<0.001) followed by HOMA-IR (X^2 =17.28, p<0.001), insulin (X^2 = 14.07, p<0.001) and adiponectin(X^2 = 9.03, p<0.001) respectively.
Conclusions: Present study demonstrated the role of adiponectin in differentiating the prediabetes from normal glucose tolerant subjects. However study warrantslarge scale study to determine the cut off value of adiponectin.

Key Word: Adiponectin, prediabetes, marker, insulin resistance.

INTRODUCTION:

Prediabetes is a metabolic state between normal glucose tolerance and diabetes.[1] It includes individuals with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT).[2] Every year, approximately 11% individuals withprediabetesprogress to type 2 diabetes.[3] Thus for reducing the prevalence of type 2 diabetes it is imperative to recognize prediabetes. Moreover recognition of prediabetes is also important for reducing thecardiovascular risks that develop even before full blown diabetes develops.[4] Conventionallyprediabetes is assessed by fasting plasma glucose and /or OGTT(3). However these tests have certain limitations.[5]Adiponectin, a secretory product ofadipose tissue, found to have insulin sensitizing property.[6] Studies found low adiponectin is a risk factor for type 2 diabetes and a marker of metabolic syndrome.[7,8] Present study thus aimed to find out the role of adiponectin as a biomarker of prediabetes as it shares the same underlying mechanism of insulin resistance.[3]

MATERIAL AND METHODS:

This cross sectional study was carried out in the department of Biochemistry, Basic Medical Sciences Institute, Jinnah Post Graduate Medical Centre, Karachi, during January to August 2008. Subjects were recruited through various diabetes screening camps. Screening schedule was distributed through different local general practitioners clinics. A day prior to screening, subjects were recruited.Subjects taking any medications that can affect blood glucose or having history of endocrine disorder like diabetes or thyroid diseases were excluded from the study.

At the time of recruitment all the participants were explained and provided a detail information leaflet about study objectives, procedures and the risk and benefits involved. All the participants were instructed to maintain fast of at least 10-12 hours till blood was collected in the next morning. The participants were reassured about the confidentiality of data. An informed consent was signed by each participant.

An interviewer administered questionnaire including demographic, anthropometric and clinical parameters including family and personal medical history was collected from each consented participants.

Anthropometric measurements including weight, height, waist and hip measurements were obtained using standardized techniques on the screening day.[9] Folding screens were used to isolate the subjects. Male and female subjects were stayed in separate rooms while conducting anthropometric examination. BMI was calculated using the formula, weight in kilograms divided by the square of height in meters. Blood pressure was recorded in the sitting position in the right arm to the nearest 2 mmHg with a mercury sphygmomanometer.

Six ml blood was collected after 8-10 hours of overnight fast to determine the fasting glucose, serum insulin and adiponectin concentration. A 75 gram oral glucose tolerance test (OGTT) was administered to each consented participants and a second blood sample for glucose estimation was drawn after 120 minutes. Fasting plasma glucose (FPG) and 2-h postload (75-g) plasma glucose (glucose oxidase-peroxidase method using kit no GL1012 manufactured by Randox UK) serum cholesterol (cholesterol oxidase-peroxidase amidopyrine method) serum triglycerides (glycerol phosphate oxidase-peroxidaseamidopyrine method), and HDL cholesterol (direct method, polyethylene glycolpretreated enzymes) were measured using a Hitachi-912 Autoanalyzer (Hitachi, Mannheim, Germany). LDL cholesterol was calculated by using the Friedewald'sequation[10].

Serum insulin was determined by monobind insulin microplate ELISA kit no 2425-300 manufactured by MonobindInc, USA. Serum adiponectin was determined by Biosourceadiponectin ELISA kit reference no. KAPME 09 manufactured by Biosource diagnosis Belgium.

A total of six hundred and eight subjects, e" 30-50 years, were screened through fasting plasma glucose and two hour OGTT. Based on the results of above tests, subjects were categorized according to ADA and WHO classification of diabetes into NGT, IFG, IFG with IGT, IGT and diabetic group. Individuals having impaired fasting glucose (e"110-d"125 mg/dl) and /or impaired glucose tolerance (e"140-d"200 mg/dl) were grouped into prediabetes group. Of total prediabetic participants (n=83) serum of forty randomly selected subjects from prediabetes group were analyzed for adiponectin estimation. Age and sex matched subjects (having IFG d"110 mg/dl and/or two hour OGTT d"140 mg/dl) were selected as control group (n=40).

STATISTICAL ANALYSIS:

As the reference value of adiponectinfor this population is not established, we take the mean values of the control subjects as a reference.The number of subjects in both the control and prediabetes groups was categorized into two groups depending on their individual value above or below mean value of the

TABLE # 1

Number of subjects having less than and greater than mean values of FPG, 2 hour plasma glucose, lipid profile, adiponectin, insulin and HOMA-IR on the mean values of normal control

			CONTROL n = 40		PREDIABETES n = 40		
S.NO	**PARAMETERS**	**MEAN VALUES**	**No. of subjects having value more than mean**	**No. of subjects having value less than mean**	**No. of subjects having value more than mean**	**No. of subjects having value less than mean**	**CHI SQUARE**
1.	Fasting plasma glucose (mg/dl)	90	21	19	29	11	3.14 N.S
2.	2 hour plasmaglucose (mg/dl)	112	23	17	40	00	21.58**
3.	Total cholesterol (mg/dl)	169	19	21	30	10	6.37*
4.	Triglyceride (mg/dl)	138	18	22	28	12	5.12*
5.	HDL-c (mg/dl)	37	23	17	28	12	1.35 N.S
6.	LDL-c (mg/dl)	104	19	21	25	15	1.81 N.S
7.	Insulin (IU/l)	11.0	18	22	34	06	14.07**
8.	Adiponectin (µg/ml)	11.4	17	23	05	35	9.03**
9.	HOMA-IR	2.48	16	24	34	06	17.28**

control subject. A chi-square test(X^2) statistics is applied to reject the null hypothesis that adiponectinmay not be used as biomarker for prediabetes. All p-values presented two tailed. The statistical tests were considered significant at the level < 5%. All the statistical analyses were performed using SPSS 13.0 software.

RESULTS:

Table 1 showed the role of various biochemical parameters in the assessment of prediabetes.Mean values of all the variables of the control subjects were taken as a reference. Based on their individual values, above or below the mean value, participants of each group was further categorize into two groups. Subjects having values equal to, or above the mean values for fasting plasma glucose, 2 hours plasma glucose, total cholesterol, HDL-c, LDL-c, insulin and HOMA-IR and values less than mean for adiponectin were analyzed through Chi-square (X^{22}) test.

For all measurements, two hour plasma glucose resulted in the best discrimination ($X^2 = 21.58$, $p<0.001$) followed by HOMA-IR($X^{22} =17.28$, $p<0.001$), insulin ($X^2 = 14.07$, $p<0.001$)and adiponectin($X^2 = 9.03$, $p<0.001$) respectively.

DISCUSSION:

Prediabetes is a metabolic state in between NGT and diabetes. It includes IFG and/or IGT subjects.[2]Prediabetes is assessed by using either fasting plasma glucose (FPG) and/or oral glucose tolerance test (OGTT).[3]However these tests have certain limitations. Though, fasting plasma glucose (FPG) test is more convenient to the patients, more reproducible, less costly and easy to administer than 2 hour oral glucose tolerance test (OGTT) and able to detect diabetes and impaired fasting glucose but it misses impaired glucose tolerance.[11] It is also found that some individuals with a normal fasting plasma glucose (FPG) have impaired glucose tolerant (IGT) or diabetes if a 2 hour oral glucose tolerance test (OGTT) is performed. But a fewer people with a normal 2 hour oral glucose tolerance test (OGTT) have IFG or diabetes.[12] Nearly 60% of subject with impaired glucose tolerant (IGT) have normal fasting plasma glucose (FPG) level.[13] Therefore fasting test alone does not detect many subjects at risk for diabetes on the basis of hyperglycemia.[14] Conversely oral glucose tolerance test (OGTT) has an advantage to detect more cases of glucose intolerance and undiagnosed diabetes than FPG test.[12] However an oral glucose tolerance test is widely presented as a burden for the patients, it has poor reproducibility and causes discomfort, inconvenience and expense. Therefore this test is not encouraged for use in usual clinical practice. On the other hand, random plasma glucose estimation lacks specificity and sensitivity.[13] Therefore screening for both diabetes and prediabetes has been repressed by uncertainty over which test to use. After discovery of adipose tissue as an endocrine organ, a number of hormones have been identified that effects on a variety of target tissues.[15,16]Adiponectin is one of the adipocyte secreted hormone.[17] Its secretion decreased in insulin resistance states.[18] Low levels of adiponectin were strongly implicated in the development of insulin resistance in a mouse model of both obesity and lipoatrophy and increased insulin resistance, improved, when adiponectin was administered.[19] Therefore we postulated that adiponectin could be used to identify the glucose dysregulation state like prediabetes as it has a central component of insulin resistance. Results of our study clearly demonstrated the ability of adiponectin in differentiating the prediabetic state from normoglycemic status. Excluding the discriminative ability of 2 hour oral glucoses test ($X^2=21.58$, $p<0.05$) that was the basis of categorizing the subjects into NGT and prediabetes in the present study, HOMA IR, insulin and adiponectin has the capability to differentiate the subjects into two category i.e NGT and prediabetes. However estimation of insulin itself also has certain limitations. First, secretion of insulin is pulsitile[20]. Second, there are situations where insulin levels are declined due to glucotoxicity especially in long standing hyperglycemic states.[21] Third, insulin estimates from serum and heparinized plasma differ, reflecting the lower insulin values found in plasma.[22] Forth, some immunometric assays that is described as "specific" for insulin showed significant cross-reactivity with proinsulins. Depends on glycemic status, this proinsulin accounts for 10 to 20% of "immunoreactive insulin" .[23] Similarly, calculation of HOMA- IR that is widely used as a research tool in epidemiological studies where invasive techniques for insulin resistance such as intravenous glucose tolerance tests are not practical also has certain limitations. HOMA IR was the index regulated by FPG and fasting plasma insulin level. It is the method to evaluate the insulin resistance where insulin secretion capacity is sustained to some extent. Secretion of insulin by beta cells of pancreas is declined by aging and glucotoxicity caused by elevated blood glucose level.[24] Calculation of insulin resistance by HOMA is not considered suitable for individual patient care as insulin results from different laboratories may vary.[25] Further more for the original model of HOMA estimation, three blood samples must be collected 5 min apart to account for insulin pulsatility and the use of the mean insulin concentration from three samples is advisable.[26]Practically this causes inconvenience and discomfort to the patients. In contrast estimation of adiponectin has several advantages. With discovery of adipokines and after elucidation of their mechanism we have entered in an era where insulin resistance may be seen from the view point of adipocytokine, which are the factors that are considered to cause insulin resistance. Adiponectin informs about the energy abundance in the body and its secretion is neither regulated nor influenced by the glycemic status of the individuals, render this hormone a suitable candidate for assessment of prediabetes.

LIMITATIONS:

With limitations of study design, patient's selection and small sample size that restrict casual relation and external validity respectively, findings of our study clearly demonstrated the ability of adiponectin in differentiating the NGT from prediabetes. However study warrants large scale study to generalize our findings and to determine the cut off value of adiponectin to differentiate NGT from prediabetes.

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