Detecting Insulin Resistance in Pakistani Subjects by Fasting Blood Samples

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Abstract: *Background*: Insulin Resistance has been identified as an independent risk factor for a number of chronic diseases such as diabetes and cardiovascular diseases. Thus identifying insulin resistant cases would help to better prevent the progression of these diseases in such individuals.

Objective: To identify a simple indirect method for detecting insulin resistance in our population by using fasting blood samples.

Methods: Geographical Imaging Systems was used for randomly selecting the subjects during an epidemiological survey done. For visiting the 532 households selected by geographical imaging systems, nine field teams were developed. A total of 871 subjects older than 25 years were approached by these teams out of which 867 agreed to participate in the survey. Insulin resistance was assessed in 227 normal subjects by fasting insulin, Homeostasis model assessment for insulin resistance (HOMA-IR), Quantitative insulin-sensitivity check index (QUICKI) and McAuley Index.

Results: Insulin Resistance was defined at 75th percentile cut off of insulin levels (9.25 U/mL) and at 75th percentile of HOMA-IR (1.82). The 25th percentile cut off was used for defining insulin resistance in QUICKI (0.347) and McAuley Index (6.77).

Conclusion: A common approach towards managing subjects with metabolic risk factors will help identify insulin resistance earlier by this fasting method and using insulin resistance reference values identified from simple indirect methods would be of value in such cases. However larger population based studies are needed to further define and validate these cutoff values for insulin resistance to be used for the general population.

Keywords: Insulin resistance, fasting blood levels, metabolic syndrome, Pakistani, HOMA-IR, QUICKI, McAuley index.

INTRODUCTION

Insulin Resistance (IR) is an acronym for a wide range of metabolic derangements with convincing evidence that it is an independent risk factor for a number of chronic diseases such as diabetes and cardiovascular diseases (CVD). Insulin resistance has also been suggested as the primary cause leading to the clustering of risk factors such as glucose intolerance, hypertension, elevated serum triglycerides, low serum HDL cholesterol and central obesity which together have been labeled as Metabolic syndrome (MS) [1].

Elevated insulin levels are believed to accelerate the development of atherosclerosis and is considered to be a key cause of cardiovascular pathologies in metabolic syndrome [2, 3]. High death rates from coronary heart diseases have been associated with insulin resistance and metabolic syndrome [4]. It has also been observed that reduction in

insulin resistance improves glycaemic control and favourably modifies other components of the metabolic syndrome [5]. Thus diagnosis of insulin resistance at the initial stages of a disease could be used as a effective measure to prevent unfavourable outcome, including reduction of cardiovascular morbidity and mortality.

Unfortunately reliable methods for measuring insulin resistance *in vivo* such as the hyper-insulinemic euglycemic clamp and minimal-model approximation of the metabolism of glucose (MMAMG) are time-consuming, complicated and require expensive equipment for epidemiological research as well as for clinical practice [6 -8].

For this purpose insulin resistance indices have been developed based on fasting blood samples (serum insulin and glucose levels) which are used as reference cutoffs defined for various populations [9]. The homeostasis model for insulin resistance (HOMA-IR), the quantitative insulin sensitivity check index (QUICKI) and McAuley Index are commonly used surrogate measures from these fasting values and have proven to be a reliable alternative to the glucose clamp studies [8, 10, 11]. Many studies using the simple indirect methods for the assessment of insulin

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resistance such as Homeostasis Model Assessments (HOMA), Quantitative Insulin Sensitivity Check Index (QUICKI), and McAuley index for detecting insulin resistance have been reported. The indirect methods HOMA and QUICKI indices are calculated using both the fasting insulin and fasting blood glucose levels while McAuley Index is calculated using fasting insulin and fasting triglyceride level [12-14].

Studies done in Pakistani population comparing hypertensive subjects with normal subjects have showed high levels of insulin in hypertensive subjects (p<0.001); likewise children having family history of cardiovascular disease had higher insulin levels compared to children with family history of diabetes [15,16]. Another study done in Pakistan showed that fasting insulin and cholesterol levels was significantly higher in subjects with diabetes compared to control subjects with a p value of <0.01 [17].

Although the prevalence of metabolic syndrome has been reported in rural and urban Pakistani population, no population based study measuring insulin resistance in Pakistani adults have been reported according to our knowledge [18-20]. Because of the lack of information on insulin resistance in our local population and lack of cutoff values, we decided to define the reference cut off values of insulin resistance by using indirect IR indices from our community based epidemiological study done previously [20].

MATERIALS AND METHODS

Study Area and Population

The survey was conducted from July 2004 to December 2004. Geographical Imaging Systems (GIS) was developed for Lyari Town with unique identification numbers ascribed to households and the prevalence of metabolic syndrome was studied by random selection of these households [20].

Methodology

The ethical approval for this survey was given by Institutional Review Board (IRB) of Baqai Institute of Diabetology and Endocrinology. The survey activities were divided into two phases-the household interview plus physical examination and blood sample collection, both have been described in detail previously [20]. Nine field teams visited the 532 households which were selected by GIS and approached a total of 871 subjects > 25 years of age out of which 867 agreed to participate in the survey. Anthropometric and demographic information was collected during the interview. Waist and hip circumference was measured and blood pressure taken twice by using mercury sphygmomanometer.

Laboratory Assays

All adults > 25 years were asked to undertake a 10 hours fast for blood tests (fasting blood glucose, lipid profile and insulin levels) for which blood samples were collected on weekends.

Blood samples were given by 363 persons out of the 867 adults who took part in the survey. All selected parameters of blood lipids (total cholesterol, triglycerides, HDL-cholesterol and LDL-Cholesterol) and blood glucose estimation was done using auto-analyzer Vitalab Selectra. Fasting blood glucose and lipid profile were done by GOD PAP method and CHOD PAP method respectively.

Insulin

Fasting insulin was measured by enzyme linked immunosorbent assay (ELISA) based on the sandwich principle. The specificity of antibodies or cross reactivity of the kit to the proinsulin was zero percent. The analytical sensitivity of the assay was found to be 1.76 uIU/ml.

Assessing Insulin Resistance

Insulin resistance was assessed in 227 normal subjects by calculating HOMA, QUICKI and McAuley Index indices as follows:

HOMA-IR

HOMA was developed by Matthews as a method for estimating insulin sensitivity from fasting serum insulin and fasting plasma glucose [11]. The non-linearity of the model precludes an exact algebraic solution, but estimations are possible by using mathematical approximations. The model has been incorporated into a simple MS-DOS-based computer program that allows rapid and more accurate determinations of HOMA-IR as shown by Matthews [11].

HOMA-IR = Insulin (Iu/ml) × glucose (mmol/l)/22.5

Low HOMA values indicated high insulin sensitivity, whereas high HOMA values indicated low insulin sensitivity while the 75th percentile of HOMA-IR was used as a cut-off for insulin resistance.

QUICKI

It is the reciprocal of HOMA-IR, known as quantitative insulin sensitivity check index (QUICKI) and increasingly used. The quantitative insulin sensitivity check index (QUICKI) is derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose:

QUICKI = 1 / (log(fasting insulin μU/mL) + log(fasting glucose mg/dL))

This index correlates well with glucose clamp studies (r = 0.78), and is useful for measuring insulin sensitivity (IS), which is the inverse of insulin resistance [12]. It also can be obtained from a fasting blood sample, and is the preferred method for certain types of clinical research.

McAuley Index

Recently, McAuley *et al.* proposed another index, which uses fasting insulin and triglyceride values, to be a strong predictor of clamp-derived Insulin Sensitivity [8,9].

McAuley Index = Exp [2.63-028 ln (Insulin in Mu/l)-0.31ln (triglyceride in mmol/l)

Statistical Analysis

Characteristics of the subjects according to gender were analyzed using an independent sample t-test. Data was presented as quartiles of fasting insulin, HOMA-IR, QUICKI and McAuley index to observe the various percentiles of insulin sensitivity and determine insulin resistance according to defined standard protocols.

The statistical analysis was conducted using SPSS for Windows (version13, SPSS Inc., Chicago, IL, USA), and p<0.05 was considered statistically significant.

RESULTS

Subjects with fasting blood sugars ≤ 100 mg/dl were taken as having normal glucose tolerance. A total of 227 normoglycemic subjects (70 men and 157 women) were selected from the survey population.

General characteristics of the study participants are shown in Table **1**. Females were significantly younger than males with lower waist circumference but higher Body Mass Index (BMI). Mean total cholesterol and LDL cholesterol was higher in females compared to males. Mean fasting insulin and HOMA-IR was also higher in females while other indices of insulin resistance were not much different in males and females. Males had significantly higher blood pressure compared to females.

The 75th percentile cut off was used as a value for defining insulin resistance for fasting insulin levels (9.25 U/mL) and for HOMA-IR (1.82) while the 25th percentile was taken as cut off for defining insulin resistance according to QUICKI (0.347) and for McAuley Index (6.77) as shown in Table **2**.

DISCUSSION

In this study females had higher fasting insulin levels compared to males; internationally there are contrasting studies about gender based insulin differences with some showing higher insulin resistance in one gender compared to the other [21].

Researchers have suggested that a fasting insulin level at 75th percentile cutoff is accurate at predicting insulin resistance in normal non diabetic population in some studies [4,9]. Fasting insulin levels at 75th percentile was thus used as a cut off value in this study and this value lies within the range observed in other studies [4,9,22].

In 1985 Matthews was one of the pioneers to define HOMA-IR as a simple and reliable method for estimating insulin sensitivity from fasting plasma glucose and insulin levels [11]. HOMA-IR values between 1.21 and 1.45 were reported for normal subjects by Matthews [11]. Many large population-based studies have used HOMA-IR to assess insulin sensitivity and reported HOMA-IR to be around 2.6 on the basis of the 75th percentile [4,10,13,14]. However lower HOMA-IR values have been observed in south asians with Indian study reporting HOMA-IR to be 1.93 at the 75th percentile while in our study it was observed to be even lower at 1.82 [23].

Other researchers suggested QUICKI to be a better surrogate measure of insulin resistance than HOMA-IR [12]. The 25th percentile value of QUICKI was 0.347 in our study which lies within the range reported for normal populations (0.33-0.372) by other researchers [4, 24].

It has been suggested by some that incorporating triglycerides in asian subjects increases the likelihood of identifying insulin resistance [25]. The index proposed by McAuley for the diagnosis of insulin resistance incorporates triglycerides in its formula as mentioned earlier [9]. Thus we calculated McAuley index on the basis of the 25th percentile and in our study the cutoff of McAuley index was 6.77. We have calculated the cut off values for defining insulin resistance in our population by using the simple fasting blood levels of insulin, glucose and triglycerides.

However our study had a few limitations. Firstly, the diagnosis of insulin resistance was based only on a single test of fasting blood glucose and insulin levels. Hyperinsulinemic euglycemic clamp studies were not

Table 1.   General Characteristics of the Study Subjects

Subject	Male Mean ± SD n = (70)	Female Mean ± SD n = (157)	P Value	Total Mean ± SD n = (227)
Age (yr)*	43.10 ± 12.95	36.87 ± 11.40	**< 0.000**	38.79 ± 12.21
Body Mass Index (kg/m^2) **	22.35 ± 4.10	25.09 ± 6.27	**0.002**	24.27 ± 6.07
Waist Circumference (cm)	87.42 ± 12.84	85.31 ± 14.09	0.288	85.96 ± 13.72
Systolic Blood Pressure (mmHg)	127.94 ± 19.95	121.73 ± 17.14	**0.033**	123.59 ± 19.55
Diastolic Blood Pressure (mmHg)*	84.70 ± 12.11	77.70 ± 12.87	**< 0.000**	79.79 ± 13.02
Cholesterol (mM/L)	175.06 ± 44.02	178.72 ± 44.85	0.581	177.61 ± 44.53
Triglyceride (mM/L)***	160.58 ± 123.27	128.90 ± 66.33	**0.018**	138.39 ± 88.25
Low Density Lipoprotein (mM/l)	108.22 ± 30.15	114.48 ± 32.56	0.181	112.59 ± 31.91
High Density Lipoprotein (mM/l)***	38.65 ± 11.52	43.48 ± 12.52	**0.009**	42.04 ± 12.40
Glucose (mM/l)	80.80 ± 8.82	79.94 ± 10.63	0.556	80.21 ± 10.10
Insulin (μU/ml)	7.60 ± 2.78	8.40 ± 4.50	0.178	8.15 ± 4.05
HOMA-IR	1.48 ± 0.58	1.61 ± 0.90	0.258	1.57 ± 0.81
QUICKI	0.36 ± 0.02	0.36 ± 0.03	0.889	0.36 ± 0.03
McAuley	7.09 ± 1.33	7.37 ± 1.48	0.138	7.29 ± 1.44

* P-value < 0.001   ** P-value < 0.005   *** P-value < 0.05.

performed to correlate the findings of indirect methods of insulin resistance with the gold standard test in this case. However, since WHO has suggested using the 75th percentile cutoff of insulin for epidemiological studies, this was also used in our study [26]. Although the sample size was small and therefore generalizing the cutoff values for Pakistani population have to be done with caution, the randomization of the sample was done by GIS which helps to reduce the bias in sample selection. Thirdly, although insulin assays can vary considerably depending on cross-reaction with proinsulin, in this study human insulin-specific radioimmunoassay was used which has no significant cross-reactivity with proinsulin thereby minimizing the interference by proinsulin [27].

Table 2. **Quartiles of Fasting Serum Insulin, HOMA-IR, QUICKI and McAuley Index**

	25th Percentile	**50th Percentile**	**75th Percentile**
Insulin	5.95	7.8	9.25
HOMA-IR	1.087	1.454	1.823
QUICKI	0.347	0.359	0.376
McAuley Index	6.77	7.046	7.33

Determining cutoff values of IR by indirect measures could help in identifying insulin resistant subjects in clinical practice on account of their simplicity and clinicians may be able to use this simple test as an initial screening tool to identify such subjects in the future. In such cases clinicians may intervene earlier in insulin resistant subjects to prevent the development of type 2 diabetes, hypertension and cardiovascular diseases in such subjects. However since the values are not universally uniform and applicable because of the ethnic variability of values studied in different populations we need to have specific population based studies. This was such an attempt to help to identify the cutoff values of insulin resistance in Pakistani population. Other studies have shown that when compared with the insulin sensitivity value obtained by the MMAMG method the sensitivity of McAuley was observed to be significantly higher than that of HOMA-IR or QUICKI [4,9]. However since HOMA-IR has been most widely studied as an indirect method for IR and has thus been considered by many researchers to be a good measure of IR [4,7].

Although determining insulin resistance by indirect methods is difficult due to the variability of results, but the cut off values of IR determined can be used as a measure of insulin resistance in Pakistani adults.

It is hoped that a common approach towards managing subjects with metabolic risk factors by using a single cutoff value will help save time and improve clinical assessment by identifying such cases of insulin resistance earlier. Secondly the clinical focus may shift from identifying the various risk factors separately towards identifying insulin resistance by using one reference values measured from these simple indirect methods.

However larger population based studies are needed to further define and validate these cutoff values for insulin resistance in this south asian population.

CONFLICT OF INTEREST

Declared none.

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