Establishment of Reference Interval for Homeostasis Model Assessment of Insulin Resistance in Healthy Adult Males: a Pilot Study in Alexandria Governorate, Egypt

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Abstract

Objective: Homeostasis model assessment of insulin resistance (HOMA-IR) is a simple and practical method for estimation of insulin resistance (IR), but a reliable reference interval (RI) of HOMA-IR is needed to deduce the threshold level to define IR. This RI has been controversial in different populations and even more divergent when considering factors like age, gender and body mass index (BMI). This pilot study aimed to establish RI for HOMA-IR in Alexandria governorate, Egypt based on the EP28-A3c document from the Clinical and Laboratory Standards Institute (CLSI).

Materials and Methods: The reference sample population included 308 healthy nondiabetic normotensive adult men aged 20-69 years with BMI 18.7-29.4 kg/m^2. Serum glucose and insulin were measured by glucose oxidase method and by electrochemiluminescence immunoassay respectively, and then HOMA-IR was calculated. The data was analyzed by non-parametric statistical methods; the values were log transformed, then outliers were truncated, and finally the reference values were constructed using 2.5th and 97.5th percentiles as lower and upper reference limits.

Results: There was no significant difference in HOMA-IR according to age or BMI; thus, the RI was established from the whole participants and it was 0.4-3.5, and subsequently values above 3.5 can be suggestive of IR status.

Conclusions: Although the reported RIs are based on recommended standards, larger scale studies including female subjects are warranted to enable the adoption of such values in laboratories in Alexandria.

Keywords: Reference interval, Homeostasis model assessment of insulin resistance, Clinical and laboratory standards institute.

Introduction

Insulin resistance (IR), defined as impaired sensitivity to metabolic actions of insulin, is associated with a number of pathological conditions; impaired glucose tolerance, type 2 diabetes (1), cardiovascular diseases (2) , and even cancer (3). The gold standard tool for its assessment is the hyperinsulinemic-euglycemic clamp which is

time-consuming, labor-intensive, and expensive (4). Still, it can be evaluated by various indices such as the quantitative insulin sensitivity check index, the oral glucose insulin sensitivity index, as well as other indices using an oral glucose tolerance test (5).
A simpler and more practical method to measure IR is the homeostasis model assessment of insulin resistance (HOMA-IR) which shows good concordance to the clamp technique. HOMA-IR is derived from fasting glucose and insulin levels, where higher values represent greater degrees of insulin resistance (6). Such interpretation is dependent on reliable reference interval which is defined by Ceriotti as "an interval that, when applied to the population serviced by the laboratory correctly includes most of the subjects with characteristics similar to the reference group and excludes the others" (7).
Perhaps some may argue to the continuous need for establishment of RI but this is justified by several reasons. First; appearance of new biomarkers for which RI has not yet been determined. Second; RI in-use, were studied and established primarily for western populations and should not be taken for granted to serve other populations. Lastly; currently used RI should be verified or validated in view of modern laboratory instrumentation and guidelines (8).
Therefore, this study aimed to establish reference interval for HOMA-IR in Alexandria governorate according to the EP28-A3c (formerly C28-A3) document of the Clinical and Laboratory Standards Institute (CLSI)/National Committee for Clinical Laboratory Standards (NCCLS) (9).

Materials and Methods

Selection of healthy reference individuals using appropriately defined criteria

This cross-sectional study was conducted on healthy men from Alexandria Governorate, the second largest city in Egypt, which extends about 32 km along the coast of the Mediterranean Sea in the north central part of the country. The participants were recruited from the community between April 2010 and August 2013 according to guidelines of the international federation of clinical chemistry (IFCC) (10). The EP28-A3c recommends a minimum sample size of 120 reference values for each reference population or subclass.
The inclusion criteria included: (a) men with stable healthy lifestyle; (b) fasting blood glucose level < 5.6 mmol/L and no previous diagnosis of type 2 diabetes mellitus; (c) BMI < 30 kg/m^2 because IR is strongly associated with obesity.
Exclusion criteria listed in the CLSI guidelines were used to exclude the following: (a) chronic disorders requiring regular medication; (b) diabetes mellitus, hypertension, coronary heart disease, stroke, tuberculosis, liver cirrhosis, cancer, hyperthyroidism, rheumatoid arthritis, systemic lupus erythematosus, chronic bronchitis, or liver/kidney failure; (c) recovering from surgery (<14 days) or acute illness; (d) blood donation in the preceding five months; (e) alcohol consumption of more than two measures (a measure is equal to 12 g of alcohol) in the preceding 24 hours; or (f) heavy smoker (>20 cigarettes/day); (g) alanine aminotransferase (ALT)>upper reference limit because of its association with insulin resistance and type 2 diabetes mellitus (11,12); (h) hepatitis B or C viral infection owing to their high incidence in Egypt, and its proved relationship with insulin resistance (13).
The procedure and purpose of the study was explained to all subjects, in their native language, and all subjects (or their legal guardians) gave their informed consent to the study, which was approved by the local ethics committee of the institute in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Demographic data was obtained from all participants, via interviews and standardized questionnaire, to retrieve information about age, physical activity, smoking, alcohol consumption, medical history, and then they were subjected to complete physical examination.

Participants were sorted according to their age into: youth (20–44 years), middle-aged (45–59 years), and elderly (60–69 years), and according to their body mass index (BMI), calculated as weight/height^2 (kg/m^2), into: healthy weight with BMI 18.5-24.9, and overweight with BMI 25-29.9. Blood pressure (BP) was measured with a standard mercury sphygmomanometer after the subjects had rested at least 10 min. Hypertension was defined as a systolic BP (SBP) of at least 140 mmHg or a diastolic BP (DBP) of at least 90 mmHg; high-normal BP was defined as a SBP of 130–139 mmHg or a DBP of 85–89 mmHg; normal BP was defined as a SBP and a DBP of 120–129 mmHg and 80–84 mmHg respectively; and optimal BP was defined as a SBP below 120 mmHg and a DBP below 80 mmHg.

Specimen collection

Following an overnight fast, eight milliliters whole venous blood samples were withdrawn from each subject. Blood sampling was performed before 9 AM. The samples were kept at room temperature for 10 minutes then centrifuged for 10-15 minutes at a minimum of 1,500 g.

Analytical procedures & QC

Standard Operating Procedures were followed during pre-analytical and analytical phases of the study. Serum was analyzed for routine clinical chemistry (glucose, lipid profile, alanine aminotransferase) enzymatically by fully automated chemistry analyzer Olympus AU400, the upper limit of alanine aminotransferase (ALT) activity was set at 38 IU/L. Serological testing for HBsAg and anti-HCV antibodies were done by ARCHITECT ci4000 system using chemiluminescent microparticle immunoassay (CMIA) technology.

Serum insulin concentration was determined using a two-site, solid phase chemiluminescent enzyme immunometric assay (CLIA) by the Immulite 1000 Automated Analyzer (Diagnostic Products Corporation). The coefficient of variation was 10.6%, the analytical sensitivity was 2 μU/ml, and there was no indicated cross-reactivity with proinsulin. HOMA-IR was calculated by the formula: fasting insulin concentration (FINS) (mU/L) × fasting plasma glucose (FPG) (mmol/L)/22.5. Linearity experiments, based on CLSI guidelines, to verify accuracy and precision were done to the laboratory quantitative parameters (14,15).

Internal QC materials were incorporated, two levels daily, and external QC materials from BioRad, twice during the analytical period, according to the manufacturers' instructions. All the daily QC runs were within ±2 standard deviations (SD) from the target values.

Statistical Analysis of Reference Values

The statistical analysis was performed using SPSS version 18. Pearson's test was used for evaluation of correlation between variables, while Kruskal Wallis and Mann-Whiteny tests were used as appropriate. A P-value < 0.05 on the two-sided test was considered statistically significant.

The CLSI guideline EP28-A3c document was followed and it included the following steps: (1) Normalization of the data for parametric analysis; after using the Shapiro-Wilk test to determine the distribution normality, if the values were not normally distributed, they were log-transformed; (2) Truncation of data for outlier rejection; the data were visually inspected for extreme values and log HOMA-IR values outside of mean ± 3SD were truncated as outliers; (3) The reference values were constructed using 2.5th and 97.5th percentiles as lower and upper limits at 95% confidence interval. Next, these values were inversely transformed and determined as the upper and lower reference limits.

Results

The relevant demographic and biochemical data were expressed as mean ± SD and median (range) (Table 1). The present study included 308 males with median age 50 years (range: 20-69) and median BMI 25.8 kg/m^2 (range: 18.7- 29.4). The median of HOMA-IR was 1.73 (range: 0.18-6.17). There was no correlation between HOMA-IR and any of the studied parameters except for fasting serum

Table 1. Demographic and biochemical characteristics of the studied participants and correlation with homeostasis model assessment of insulin resistance (HOMA-IR)

Parameter	Mean ± SD	Median (Range)	Correlation with HOMA-IR
Age (years)	46.04 ± 12.73	50 (20-69)	r= 0.032 (*P*=0.58)
BMI (kg/m^2)	25.57 ± 2.15	25.80 (18.7-29.4)	r=0.020 (*P*=0.725)
FSG (mmol/L)	4.79 ± 0.30	4.71 (4.22-5.38)	r=-0.045 (*P*=0.427)
FIN (uIU/mL)	8.36 ± 4.18	8.11 (0.86-29.1)	r=0.991 (*P*<0.001*)
HOMA-IR	1.77 ± 0.88	1.73(0.18–6.17)	----------
ALT (U/L)	23.04 ± 6.62	24 (10-35)	r=-0.012 (*P*=0.827)
TC (mg/dL)	201.10 ± 17.27	200 (125-233)	r=0.067 (*P*=0.239)
HDL-C (mg/dL)	42.35 ± 8.04	41.0 (30-67)	r=-0.061 (*P*=0.282)
LDL-C (mg/dL)	136.26 ± 18.53	136.8 (55-178)	r=0.085 (*P*=0.138)
TG (mg/dL)	112.4 ± 17.41	117.0 (68-145)	r=0.025 (*P*=0.656)
SBP (mmHg)	116.69 ± 12.7	120 (87-135)	r=-0.054 (*P*=0.343)
DBP (mmHg)	77.85 ± 5.81	80 (56-85)	r=-0.055 (*P*=0.339)

BMI, body mass index; FIN, fasting insulin; ALT, alanine aminotransferase; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triacylglycerol; SBP, systolic blood pressure; DBP, diastolic blood pressure.
r= Pearson coefficient
*statistically significant at $P \leq 0.05$

insulin (r= 0.991, *P*<0.001) (Table 1).

The studied participants were subdivided according to their age into: youth (125 subjects, 40.58%), middle-aged (124 subjects, 40.26%), and elderly (59 subjects, 19.16%). Also, they were sorted based on BMI into healthy weight (114 subjects, 37%), and overweight (194 subjects, 63%). There was no significant difference in HOMA-IR according to age (*P*=0.811) or BMI (*P*=0.932) (Table 2).

Since HOMA-IR values were not normally distributed (Shapiro-Wilk test, *P*<0.005), and there was a degree of right-sided skewness (skewness= 1.045 and kurtosis= 3.182) (Figure 1), log-transformation (log HOMA-IR) (skewness=-0.925 and kurtosis=1.272) (Figure 2) was done. The data was visually inspected for extreme values and log HOMA-IR values outside of mean ± 3SD (-0.56 and 0.93) were truncated as outliers (Figure 2). Only three values were excluded because they were lower than the value of the mean-3SD.

Then, the 2.5th and 97.5th percentiles of log HOMA-IR values were calculated which were respectively -0.4 and 0.54 at 95% confidence interval. Next, these values were inversely transformed and determined as the upper and lower reference limits (i.e. 0.4 and 3.5, respectively). Thus, HOMA-IR > 3.5 may indicate insulin resistance.

Laboratory results are simply interpreted by comparison with reference intervals (RI), thus the reliability of RI is crucial to many medical decisions based on lab tests. But establishment of RI is a tedious job, which made adoption and transference of manufactures RI, even without validation, a common practice in many labs especially in developing countries with limited resources. Such RIs are basically derived from western countries, with a major dearth in others. The present study attempted to establish a 'health-associated' RI for

Table 2. Homeostasis model assessment of insulin resistance (HOMA-IR) in the studied participants

Variable	N (%)	Mean ± SD	Median	Range	Comparison among the studied groups
Total	308	1.77 ± 0.88	1.725	0.18–6.17	
Age (years)					
Youth (20-44)	125 (40.58)	1.73 ± 0.88	1.700	0.2–6.17	H= 0.419
Middle-aged (45-59)	124 (40.26)	1.76 ± 0.76	1.781	0.29–3.88	*P*= 0.811
Elderly (60-69)	59 (19.16)	1.88 ± 1.09	1.786	0.18–5.83	
BMI (kg/m^2)					
Healthy (18.7-24.9)	114 (37)	1.76 ± 0.87	1.786	0.4–6.17	Z= -0.085
Overweight (25-29.4)	194 (63)	1.78 ± 0.89	1.702	0.18–5.83	*P*= 0.932

N: number; BMI: body mass index.
H: Kruskal-Wallis test among different age groups
Z: Mann Whitney test between healthy and overweight

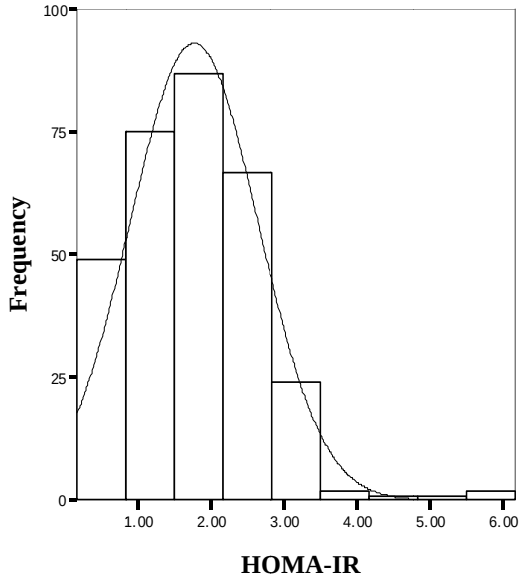

Figure 1. Frequency histogram of Homeostasis model assessment of insulin resistance (HOMA-IR).

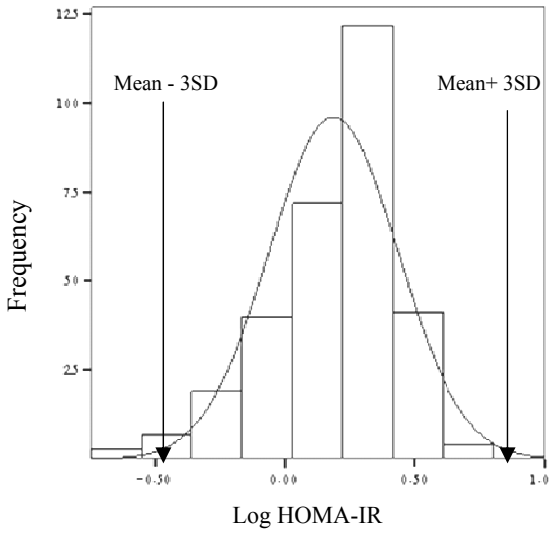

Figure 2. Frequency histogram of logarithmic transformation of Homeostasis model assessment of insulin resistance (log HOMA-IR) with arrows denoting the truncation of values outside the mean ± 3 SD.

HOMA-IR which is derived from a reference sample group who are apparently in good health.

One of the initial problems associated with the calculation of RI is lacking a universal definition of "Health"; thus, even with stringent selection guidelines there will always be some level of uncertainty or possibility that some of the participants might have subclinical disease. Another common pitfall is standardization of pre-analytical variables as well as analytical and quality control procedures (7). These are among the leading causes that resulted in announcement of CLSI EP28-A3c document in a trial to unify RI studies. Even though, some problems are encountered in HOMA-IR studies per se, such as the multifactorial etiology of insulin resistance including ethnic, racial, environmental and physiological factors, and the absence of worldwide standardized assay for insulin as well as possible inter-laboratory variation especially if antibodies cross-reacting with proinsulin are used. Besides the different statistical approaches, ROC curves or different percentile values, used in previous studies for determination of the threshold level to determine IR have led to some discrepancies even in the same population (16). Therefore it is imperative to establish reference values for HOMA-IR using standardized procedures to permit possible inter-population comparison.

The RI established from the present study, deduced from the 2.5th and 97.5th percentiles based on CLSI EP28-A3c document, was 0.4-3.5 and subsequently values above 3.5 can be suggestive of IR status. This cut-off value is higher than the currently used values in laboratories in Alexandria which vary from 2 to 2.5 and which were derived from the western literature. Actually, a variation was quite expected due to differences in ethnicity, genetic load, life style, dietary habits as well as ecological factors in our population when compared to western ones. Needless to say the use of threshold values above the 97.5th percentile has resulted in such higher values, and if a lower percentile would have been used, lower values should be expected. For example, if the 90th percentile was applied in the present study, the cut-off point would be 2.8. This highlights the necessity of establishment of region specific RIs based on standard guidelines. Future population studies should address the role of environmental and

genetic factors in the higher values of HOMA-IR in our reference population.

Several population-based studies in different geographical areas have been conducted on HOMA-IR to report the optimum cut-off point suggestive of IR. In Asian studies, a cut-off point of 2.5 has often been used (17-19), but still different values were reported in others. Consider for example, the Japanese populations where several studies have determined several upper limits for HOMA-IR of 1.7, 1.73, 1.97, 2.0 and 2.4 (20). In Tehran, it was demonstrated to be 1.775 in nondiabetic, normotensive individuals aged 25-64 (n=3,071) (16).

Few studies have been conducted on Mediterranean Caucasians baring some resemblance to our Alexandrian population. In an Italian study, Bonora et al. reported that the top quintile of the HOMA-IR, a value ≥2.77, had isolated IR in subjects with no metabolic disorders (n=888, aged 40-79 with mean BMI <25 kg/m^2) (21). Another study in Spain, suggested a higher cut-off of 3.8, using the upper 90 percentile, in subjects without diabetes (n=97, average BMI 22.2 kg/m^2) (22).

In the present study, there was no significant difference in HOMA-IR based on either differences in age or BMI. That is why there was no need for partitioning the sample reference group or developing age specific reference values; consequently, a single reference interval was deduced to the whole studied participants. On the other hand, some studies had specified certain age population. Lee et al. used interval setting and showed an upper reference limit for HOMA-IR of 4.39 among American adolescents (n=1,804 with average BMI 24 kg/m^2) (6). While in Chilean elderly population in South America (n=1,003, 20% diabetics), the cut-off point for IR was identified as the 75th percentile and it was reported as 2.57 (23).

One strength of the present study was that it strictly followed the recommendations of the CLSI guidelines for determining RI. On the other hand, the small sample size especially in the elderly and restriction to male subjects only can be considered some form of limitation.

To the best of our knowledge, this work was the first trial to report guideline-based HOMA-IR reference interval in a sample group from Alexandria governorate following CLSI EP28-A3c document. Although the reported reference intervals are based on recommended standards, it is highly recommended to perform RI studies at a larger scale including female subjects in Alexandria as well as other governorates in Egypt especially those with different environmental characteristics. This will guarantee better evaluation of insulin status, thus enabling identification of those with insulin resistance in a strategy for preventive interventions.

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