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Reference Interval of Homeostasis Model Assessment- Insulin Resistance in an Iraqi Adult Population: A Study in Nineveh Governorate

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BMI; HOMA-IR; insulin resistance; Iraq; Mosul City; Nineveh; Reference Interval.

ABSTRACT

Background: Insulin resistance (IR) is a condition in which the tissues become less responsive to insulin. IR has been linked to many diseases including Type 2 Diabetes Mellitus (T2DM), pre-diabetes, and metabolic syndrome. The most commonly used tool of IR assessment is Homeostasis Model Assessment of insulin resistance (HOMA-IR). In Iraq, there is limited data on the reference interval of HOMA-IR.

Objectives: To calculate the reference interval of HOMA-IR among a randomly screened sample of adult people in Nineveh Province, Northern Iraq.

Methods: This is a cross-sectional study that finally involved 286 apparently healthy adults (≥25 years). Data were collected in the period from the 1st of September to the end of December 2024. Weight and height were measured and body mass index (BMI) was calculated for each participant. Fasting serum insulin and glucose were tested and used to calculate HOMA-IR.

Results: The overall mean ±SD of HOMA-IR for the study population was 2.11±1.34. HOMA-IR 95% reference interval was calculated based on the 2.5th percentile and 97.5th percentile after removing outliers and was (0.53-4.32). The participants were then divided according to BMI into 3 groups. For subjects with BMI below 25 Kg/m^2, the mean HOMA-IR was 1.62±1.2, and the 95% reference interval was (0.46-3.54). For Subjects with BMI from 25-29.99 Kg/m^2, the mean HOMA-IR was 1.88±0.75 and the reference interval was (0.64-4.04), however, for subjects with BMI ≥ 30 Kg/m^2, the mean ±SD of HOMA-IR was 2.71±1.61 and the reference interval was established to be (0.49-4.75).

Conclusions: The upper end of the reference interval in our study was a bit lower than previous studies in Iraq, and comparable to other studies in the region. Higher BMI is associated with increase in the mean HOMA-IR ($p < 0.000$). The differences in the methods used to obtain HOMA-IR reference interval may lead to significantly different results.

Introduction

Insulin resistance (IR) is defined as a condition in which the tissues which are targeted by insulin become less responsive to physiological or even to high levels of insulin. IR has been linked to many pathological conditions including type 2 diabetes mellitus (T2DM), pre-diabetes, non-alcoholic fatty liver disease (NAFLD), obesity and metabolic syndrome [1]. The term “insulin resistance” was first used in 1931 by Wilhelm Falta, a physician in Vienna/ Austria, since then the understanding of this pathological condition has been growing, especially after the development of laboratory methods for insulin measurement, the first of which was introduced by Rosalyn Yalow and Solomon Berson who were awarded the Nobel Prize in physiology in1977 [2].

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Hyperinsulinemic euoglycemic clamp (HEC) is considered as the golden standard approach for the accurate measurement of IR in humans. However, this method is time consuming, invasive, expensive, and requires highly experienced technicians to manage its complicated procedure, so it is not generally feasible for research setting which involves large populations [3].

Other more practical alternative tools were proposed for assessing insulin resistance including different surrogate mathematical calculations. The most commonly used among them is Homeostasis Model Assessment of insulin resistance (HOMA-IR) [4]. HOMA-IR was developed by Mathews et al in 1985 and is still used widely for IR estimation [5].

In addition to its use as an estimator of IR, HOMA-IR was proved to be of value in predicting metabolic conditions such as T2DM and prediabetes [6]. A study in 2023 found it to be useful in predicting new onset T2DM regardless of HbA1c level, suggesting it can be used for identifying individuals at risk for developing diabetes [7].

In general, when laboratory tests are performed, a range of values is usually given to allow for interpretation of results.the “reference interval” (RI). The (RI) is usually defined as the limits within which fall 95% of the results which are derived from a well-defined reference population [8]. Sometimes the term [reference range] is used interchangeably with [reference interval]; however, the term “interval” is more accurate. A reference range is generally used to represent the difference between the upper and the lower limits of an interval [9].

The diagnostic validity of a test is largely dependent on establishing an accurate reference interval. To this day there is limited data on the reference interval of HOMA-IR in our locality, and different studies among different populations produced different results. Only few countries have recommended reference intervals for HOMA-IR in their populations [10] [11]. This research was conducted in Mosul, a major population center in Northern Iraq, aiming to bridge this gap by establishing the reference interval for HOMA-IR in our local adults’ population.

Methods

Study Design

This cross- sectional study was conducted in Nineveh Governorate, Northern Iraq, through the period from 1st. of September to 31st. of December 2024. Subjects included in the establishment of the reference interval for HOMA-IR were all adult (≥25 years) apparently healthy, with normal glycemic state (neither diabetic nor pre-diabetic). For this purpose, investigations were done to exclude states of diabetes and pre-diabetes according to the criteria of American Diabetes Association (ADA) using values of both fasting serum glucose (FSG) and glycated hemoglobin (HbA1c) [11].

The study initially screened randomly a total of 410 participants divided into seven distinct samples. The first group consisted of 67 individuals (23 men and 44 women) from the Al-Qahira quarter on Mosul’s left bank. The second sample, which consisted of 63 participants (25 males, 38 females), was drawn from the Al-Tise’en quarter, also located on the city’s left side.

For the third sample, we gathered data from 55 subjects (19 men and 36 women) in Tammuz quarter on Mosul’s right side. Similarly, the fourth group came from Al-Thawra neighborhood on the same side of the city, comprising 52 people (20 males and 32 females).

The fifth sample included 46 participants (18 men and 28 women) from Bejwanya village, situated south of Mosul City. Another 66 individuals’ sample (23 males and 43 females) was selected from Al-Hamdannyah Town, roughly 25 kilometers southeast of Mosul. The final group included 61 participants (21 men and 40 women) who were randomly recruited from among relatives accompanying inpatients in various Mosul hospitals, all of whom agreed to take part in the study. From the above sample, a total of 286 subjects finally formed the normoglycemic population for whom the reference interval of HOMA-IR was established.

Ethical Considerations: This study was approved by the Medical Research Ethics Committee/ College of Medicine, University of Mosul (UOM/COM/MREC/24-25/SEP2) and the Scientific and Research Ethical Committee of Nineveh Health Directorate (no.2024131), and written consents were collected from all participants.

Anthropometric Measurement: Height and weight, were recorded for all subjects while wearing light clothes and being shoes off. Body mass index (BMI) was calculated using the following equation [13]:

BMI= Weight (kg)/[Height(m)]2

Medical History: A data collection form was used to collect socio-demographic data like age, sex, residence, occupation in addition to past medical history, family history of diabetes, and drug history.

Specimens: A 5 ml venous blood sample was collected from every participant. Two- ml were placed in EDTA tube for HbA1c testing, while the other 3 ml were placed in a plain tube and allowed to clot. Serum was obtained through centrifugation of clotted blood samples at 4000 rpm for 5 minutes. Serum was separated into two aliquots; one was immediately tested for fasting glucose and the other was frozen at -20 °C to be tested for insulin in batches.

Laboratory Methods: All measurements were run at the Clinical Biochemistry Laboratory at the College of Medicine, University of Mosul. HbA1c was measured using Cobas 6000 analyzer where the HbA1c concentration and its percentage to total hemoglobin were determined based on the principle of Turbidimetric Inhibition Immunoassay [14]. FSG was measured using FUJI DRI-Chem NX500 chemistry analyzer based on the principle of colorimetry using commercially available kits according to the instructions of the manufacturer. The kits are basically multilayered slides containing dry chemistry ingredients [15].

Fasting serum insulin was measured using TOSOH AIA-360 analyzer (Japan) and AIA-Pack IRI kit, the test is based on the principle of immunenzymometric reaction [16]. HOMA-IR was calculated using the formula [17]:

HOMA1-IR= {[Fasting Insulin (µU/ml) X fasting glucose (mg/dl)] ÷405}

Statistical Analysis

Statistical Package for Social Sciences (SPSS), version 22 and Microsoft Excel (2019) were used to analyze the data. Descriptive statistics included calculating the mean± SD to present the continuous variables, while categorical parameters were presented as number (%). Chi-square test was used to compare categorical variables while Kruskal-Wallis test was used to compare the mean values of those continuous variables which were not normally distributed. The reference interval was calculated as the 2.5th-97.5th percentile range after proper removal of outliers following the Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. Significance of statistical comparisons was considered at p- value < 0.05.

Results

Out of 410 adult subjects initially tested for their glycemic state, 286 (102 males and 184 females) were identified as non-diabetic and non-prediabetic according to the ADA guidelines [11]. They formed our population sample to establish the reference interval for HOMA-IR in our local population. Their overall mean age was 41.3 ±11.53 yr. The mean values of FSG, HbA1c, insulin, and BMI were 88.97± 6.58 mg/dl, 5.06±0.32%, 9.05± 5.87 µU/ml, and 28.27±5.28 kg/m2 respectively. Subjects were subdivided into 3 groups based on BMI categories; < 25kg/m^2, 25- 29.9 kg/m^2, and ≥ 30 kg/m^2. The general characteristics of study subjects are presented in Table (1).

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Table 1: Basic characteristics of study subjects by BMI. Data are presented either as n (%) or mean ±SD as appropriate.

		Normal BMI (BMI < 25kg/m^2) (n= 87)	Overweight (BMI 25-29.9 kg/m^2) (n= 95)	Obese (BMI ≥ 30 kg/m^2) (n= 104)	Total (n=286)	p- value
Gender	Males	30	34	38	102	0.982
	Females	57	61	66	184	
Age (yr)		38.19±12.35	40.7±11.13	44.44±10.52	41.3±11.53	<0.0001
HOMA-IR		1.62±1.2	1.88±0.75	2.71±1.61	2.11±1.34	<0.0001
Fasting insulin µU/ml		7.47±5.36	8.53±3.44	12.08±7.03	9.05±5.87	<0.0001
FSG (mg/dl)		87.29±6.85	89.33±6.23	90.03±6.43	88.97±6.57	0.006
HbA1c (%)		5.01±0.3	5.05±0.33	5.12±0.33	5.06±0.32	0.016
BMI (kg/ m^2)		22.68±2.21	27.28±1.48	33.75±3.68	28.27±5.28	<0.0001

For establishing the reference interval of HOMA-IR, HOMA-IR values were assessed for normality of their distribution using Kolmogorov-Smirnov test. They were found to be not normally distributed (p<0.0001) and skewed to the right (skewness= 1.35), Figure (1). Log transformation of data did not alternate them to the Gaussian pattern either (p<0.0001), Figure (2). Removing the outliers could enhance the outcome, but the CSLI guidelines recommend achieving a normal distribution through transformation before removal of outliers [18].

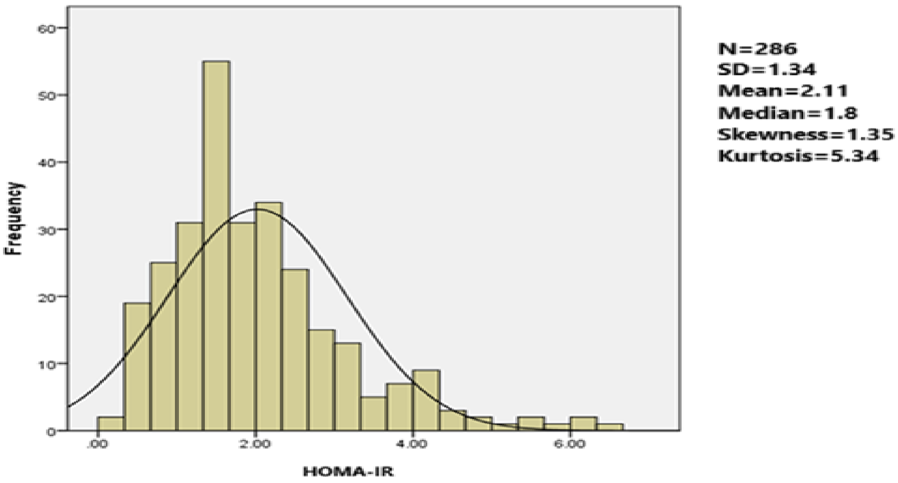

Figure 1. *Distribution of HOMA-IR values among the study population.*

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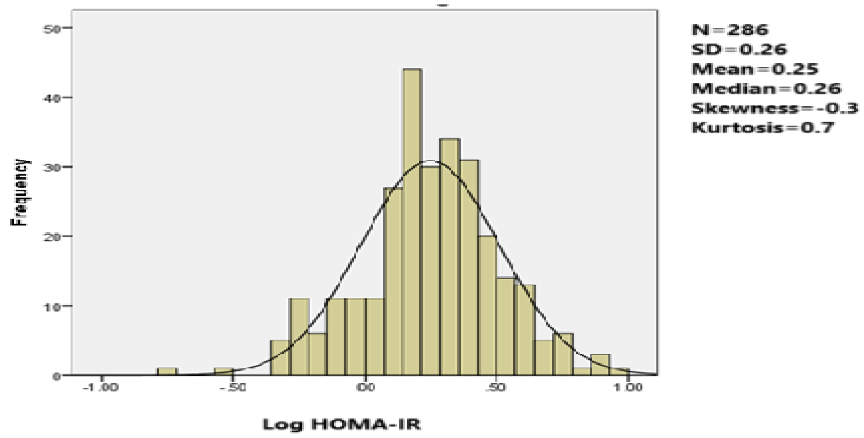

Figure 2. Distribution of HOMA-IR values among the study population after log transformation.

The 95% reference interval was calculated by taking the 2.5th and 97.5th percentiles, but first a Cox-box transformation [19] was performed by raising HOMA-IR values to the power of 0.3 to produce a set of data with Gaussian distribution, Figure (*3*). The outliers of the transformed data were detected by using Tukey's method [20].

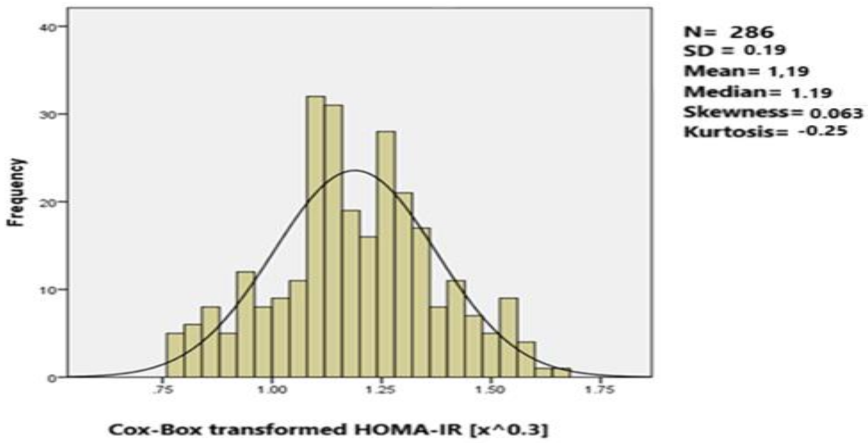

Figure 3. Distribution of Cox-Box transformed HOMA-IR values. Values were raised to the power of 0.3.

The inter quartile range (IQR) for the transformed data was calculated (the range between 25th and 75th percentiles), then all values above (75th percentile+ (1.5×IQR)) and all values below (25th percentile-(1.5×IQR)) were considered as outliers as per Tukey's method, and were removed with their subsequent original HOMA-IR values. A total of 12 outliers were detected and removed, 2 from the lower end, and 10 from the upper end. The resulting reference interval for HOMAIR was 0.53- 4.32. This interval represents a set of values within which will lay 95% of HOMA-IR values coming from apparently healthy individuals. The overall mean HOMA-IR after outlier removal was 1.96 ± 0.98.

This method of removing outliers and then calculating the reference interval follows the CSLI guidelines recommendation for tests values which are not normally distributed [18]. Results of Kolmogorov- Smirnov test before and after Cox-box transformation are shown in Table (2).

Table 2. Results of Kolmogorov-Smirnov test of normality before and after Cox-Box transformation.

	Kolmogorov-Smirnov test's Significance	Skewness	Kurtosis
HOMA-IR	<0.0001	1.35	5.34
Log –HOMA-IR	< 0.0001	-0.3	0.7
(HOMA-IR)^0.3	0.2	0.06	-0.25

Based on the known association between obesity and insulin resistance, subjects were subdivided into 3 groups and the reference interval of HOMA-IR was calculated for each BMI group, as follows:

Subjects with BMI below 25 Kg/m^2: eighty-seven (30 males and 57 females) subjects were in this category. The mean ±SD for HOMA-IR was 1.62±1.2. The reference interval was established to be (0.46-3.54).

Overweight subjects (BMI from 25-29.9 Kg/m^2): ninety-five (34 males and 61 females) subjects were in this group. The mean ±SD for HOMA-IR was 1.88±0.75, thus the reference interval was calculated to be (0.64-4.04).

Subjects with obesity (BMI ≥ 30 Kg/m^2): these are 104 subjects (38 males and 66 females). The mean ±SD of HOMA-IR was 2.71±1.61 and the reference interval of HOMA-IR was (0.49-4.75), Table (3).

HOMA-IR reference interval was not calculated for different age groups since some age groups did not have adequate number of subjects as recommended by the CSLI guidelines for calculating reference intervals [18]. HOMA-IR reference interval by sex is shown in (Table 4)

The distribution of HOMA-IR across BMI groups, and sex groups are shown in Figures (4, 5).

Table 3. HOMA-IR descriptive statistics and reference intervals according to BMI categories.

BMI classes (Kg/m^2)	N (%)	Median	SE mean	Reference Interval
<25	87	1.43	0.13	0.46-3.54
25-24.9	95	1.78	0.08	0.64-4.04
≥30	104	2.25	0.16	0.49-4.75
Total	286	1.8	0.08	0.53- 4.32

Table 4. HOMA-IR descriptive statistics and reference intervals according to sex.

Sex	N (%)	Median	Std. Error of Mean	Reference interval
Male	102	1.89	0.147	0.59-4.64
Female	184	1.66	0.091	0.54-4.19
Total	286	1.8	0.08	0.53- 4.32

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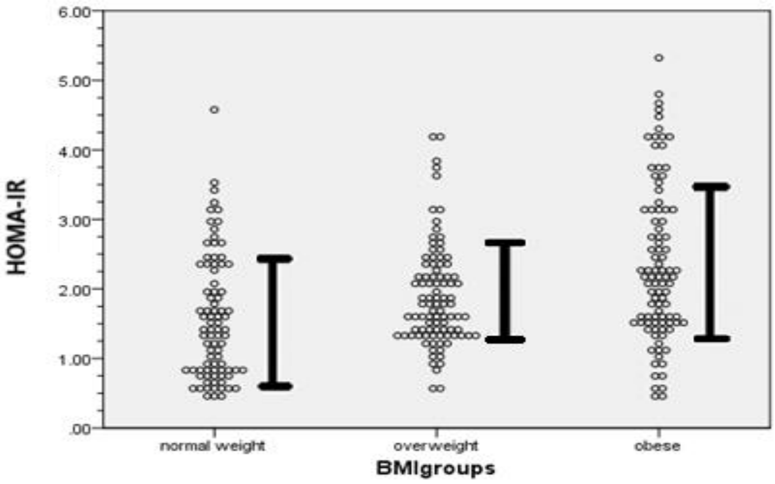

Figure 4: HOMA-IR distribution across BMI groups. Bars represent mean ±SD

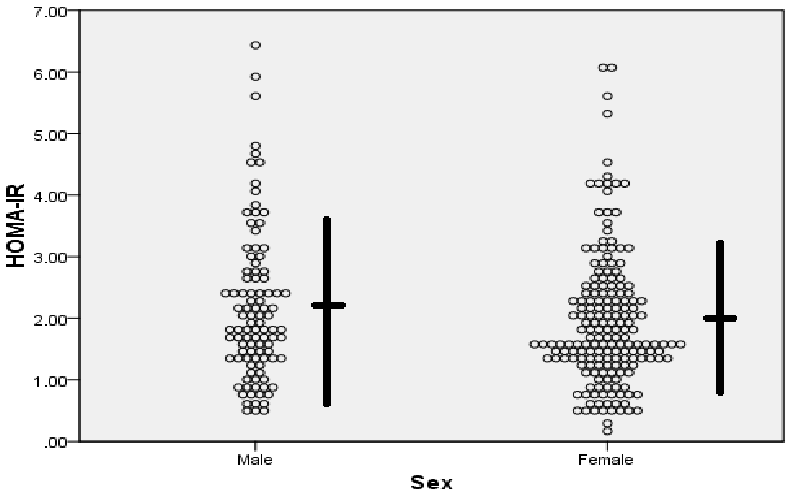

Figure 5. HOMA-IR distribution by sex. Bars represent mean ±SD

Discussion

Insulin resistance has been linked to different metabolic disorders, including T2DM, prediabetes, metabolic syndrome, and others [21]. Ideally, direct procedures like the hyperinsulinemic euglycemic clamp or intravenous glucose tolerance test should be used to assess IR, but due to their invasive nature and complicated setting, several surrogate indexes have been introduced including HOMA-IR [4].

Insulin sensitivity is influenced by many factors including obesity, physical activity, age and ethnicity. Studies have also shown that glucose tolerance is reduced by age and by increased body fat mass [22].

In this study, the reference interval for HOMA-IR among the healthy normoglycemic Iraqi adult population in Nineveh Province appears to be a bit wider than that revealed by other researches in other parts of the world. In Japan, a study in 2011 found the reference interval of HOMA-IR to range between 0.4 and 2.4(10). Another study in Brazil in 2023 revealed a reference interval value of 0.39-2.86 for females, 0.38-2.81 for males, and 0.39-2.86 for overall population. These figures appear to be close to the Japanese study, but the higher end is narrower than the finding of our study [23]. It is worth mentioning that both the Japanese and the Brazilian studies used the log transformed HOMA-IR data. The methodology used in obtaining reference intervals greatly affect the results. There are different ways of removing outliers, and different ways to normalize the data which are not normally distributed. Some researchers use a direct range from the lowest to the highest limits, others use the 5%-95% range, all this can lead to narrower or wider ranges.

For experiment purposes, we used the log transformation method for our data and we applied the mean ± 2SD method and the 2.5^{th}, 97.5^{th} percentile methods on the Log transformed HOMA-IR data. Then we converted the results back to the original HOMA-IR values. The resulting reference intervals were (0.78-2.12) and (0.76-2.16) respectively, much closer to the findings of the Japanese and the Brazilian studies.

The use of log- transformed data was not recommended in our study since it did not follow the Gaussian pattern of distribution as mentioned earlier, and hence the (2.5^{th}, 97.5^{th}) method was alternatively employed after removal of outliers using the Cox-Box transformed data.

In a Polish study published in 2020, researchers collected data from 130 young participants (ages 18-31 years) and found the HMOA-IR's reference interval to be 1.14-4.0. However, they used the (5^{th}, 95^{th}) percentiles, which makes the comparison difficult without the complete data [24].

In another study conducted in Iran in 2022, HOMA-IR data was collected from 6637 individuals aged 18 to 70 years [25].

They found the mean HOMA-IR to be 2.11±0.99 and HOMA-IR reference interval (using the 2.5^{th}, 97.5^{th} percentile method) to range between 0.66 and 4.5, which is slightly higher at the lower end and lower at the upper end compared to the those we revealed. The mean (SD) of BMI was 26.5±3.8 compared to 28.27±5.28 in our study. This may explain the difference at least in part.

In our study the reference interval of HOMA-IR showed notable differences across BMI groups. Unfortunately, the Iranian study did not present the HOMA-IR data by BMI categories, so we couldn't confirm this assumption.

A study in Baghdad/Iraq in 2023 found HOMA-IR among apparently healthy adults to range from (0.46-5.37) with the mean of 2.37±1.18. This range is wider than ours (0.53- 4.32), but the mean HOMA-IR in our study (2.11±1.33) is comparable. This perhaps suggests that a different method might be employed in the calculation of the HOMA-IR's reference interval, which is, unfortunately, not mentioned in Baghdad's study [26].

The differences in the reference interval in this study compared to others could be due to differences in the sample size.Up to our knowledge no other recent studies about the reference interval of HOMA-IR in Iraq were encountered. However, several studies calculated the mean HOMA-IR in apparently healthy individuals in Iraq. A small cross- sectional study was published by Al-Naemi in 2022, and included 90 apparently healthy individuals in Mosul/Iraq [4]. It found the mean HOMA-IR to be 1.4±0.76. This is significantly lower than the mean value revealed in the current study. The mean BMI in that study was 26.3±5.23kg/m^2 compared to 28.27±5.28 kg/m^2 in ours. Moreover, that study excluded subjects with family history of diabetes, which could also be a part of difference, since genetic factors may be linked to increased insulin resistance in some people [27].

In conclusion, the reference interval of HOMA-IR among adult Iraqi subjects with normal glycemic state is 0.53- 4.32 with notably different intervals upon BMI categorization. The methods of calculation employed by different researchers worldwide may lead to significantly different results. Further studies involving a larger sample size are needed to produce a more robust value of HOMA-IR's reference interval that can be applied nationally in Iraq.

Study limitations

HOMA-IR reference interval was not calculated for different age groups since some age groups did not have adequate numbers of subjects for calculating reference interval, Socioeconomic status and physical activity levels were not considered due to difficulty in obtaining accurate information. All this needs to be considered in future work.

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Conflict of interest

Authors declare that they don’t have any conflict of interest.

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