ORIGINAL ARTICLE

Diagnosis of Hyperinsulinaemia in a Normoglycaemic Healthy Indian Population - Developing Ethnic Reference Ranges

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Abstract

Context: Reference limits for diagnosing hyperinsulinaemia are currently derived from non-Indian cohorts and have not been validated in Indians even though it is acknowledged that different patterns of insulin secretion are seen across ethnicities.

Aims: To develop ethnicity specific reference limits for insulin levels in a normoglycaemic healthy Indian cohort in order to derive a clinical cut off for hyperinsulinaemia as an effective screening tool for predicting future risk of metabolic and cardiovascular disease.

Settings and Design: Prospective analysis of plasma insulin levels in healthy normoglycaemic volunteers availing diagnostic facilities at a central reference laboratory in Mumbai.

Methods and Material: 122 normoglycaemic males between 19-73 years and 126 females between 19-55 years of age were selected based on a screening questionnaire as per the Clinical Laboratory and Standards Institute (CLSI) guidance document for deriving reference ranges. Fasting insulin levels were analysed using a Chemiluminescent Microparticle Immunoassay platform and derived results were analysed to determine reference limits for insulin.

Statistical analysis used: A non-parametric method of statistical analysis was used to determine the 2.5 and 97.5% limits with 90% confidence intervals.

Results: Reference range for insulin in a normoglycemic Indian cohort was derived as 2.7-17 uIU/ml which established 17 uIU/ml as the clinical cut off for diagnosing hyperinsulinemia in healthy Indians.

Conclusions: Reference limits for insulin in normoglycemic Indians needs to be revised to 2.7-17 uIU/ml. Clinical cut off for hyperinsulinaemia needs to be lowered to 17 uIU/ml from currently used cut offs which range from 25-31 uIU/ml.

Key Messages: Reference limits currently used for diagnosing hyperinsulinemia in healthy normoglycemic adults need to be revised and made specific for different ethnicities. In Indians the upper limit of the normal reference range for insulin levels needs to be brought down to 17 u IU/ml from the existing 25-31 u IU/ml. This modified cut off would help clinicians identify apparently healthy individuals who may need to be screened for a future risk of metabolic and cardiovascular disorders.

Introduction

Epidemiological studies in India since 1986 have shown that the incidence of diabetes is high in our country and ethnic differences across geographies are known to affect both the prevalence and manifestations of diabetes.¹ ² Differential patterns in insulin secretion have also been observed across various ethnic groups. Proof of this concept has been well documented in previous studies of Indians living in India or in foreign lands which have shown characteristic features of hyperinsulinaemia and insulin resistance (IR).³ ⁴ Since hyperinsulinaemia is usually the compensatory response to
insulin resistance, closely monitoring both fasting insulin and glucose is important in identifying people who have a high risk predisposition for the development of diabetes.

Many citations have also spoken upon the long term link of hyperinsulinaemia to dysglycaemia, impaired glucose tolerance, hirsutism and PCOS. In children and young Asian Indians a high intake of n-6 PUFA is correlated with fasting hyperinsulinaemia and in adults high carbohydrate meal consumption was reported to cause hyperinsulinaemia. More recent publications have also indicated that high baseline fasting insulin levels are independent determinants for the future development of Metabolic Syndrome (MS).

In spite of hyperinsulinaemia being a precursor to several of the above mentioned disease states and a predominant factor given the dietary habits of Asian Indians, recent citations have pointed out that the absence of population specific reference ranges for hyperinsulinaemia is glaring when compared with that for blood glucose where cut offs for abnormal levels have decreased over the years, as it became evident that far lower levels of glycaemia were a health hazard. Similarly, for basal insulin measurements to become a useful diagnostic tool in a healthy normoglycaemic population, clinically relevant reference limits of hyperinsulinaemia need to be determined in different ethnicities.

In this work we attempted to derive reference values for fasting plasma insulin in a normoglycaemic cosmopolitan Indian cohort based on current laboratory medicine guidelines. Our hypothesis was that these derived reference values may help increase the accuracy of interpreting laboratory results of this parameter in Indians as to our knowledge currently used reference ranges from standard text books and manufacturers’ kit inserts have not been formally validated in an Indian setting.

Subjects and Methods

Material and Methods

Ethics

Participation was voluntary and consent was obtained from all volunteers. Risk analysis, patient management and confidentiality requirements were followed as per NCCLS guidelines. No formal ethics committee permission was obtained as data analysis was performed on tests requested for by participants themselves during the routine course of their health check-up and no additional investigations were performed solely for the purpose of this work.

Study Design

Selection and Description of Participants

Participants were chosen from among healthy volunteers availing diagnostic facilities at Metropolis Healthcare Pvt. Ltd., a College of American Pathologists and ISO 15189 accredited central reference laboratory at Mumbai from 2009-2010. Selection of participants was made as per standard procedures described in the clinical and laboratory standards institute (CLSI) guidelines, formerly NCCLS. This document is based on the publications of the expert panel on theory of reference values (EPTRV) of the International federation of clinical chemistry (IFCC) and the standing committee on reference values of the international council for standardisation in haematology (ICSH).

Total sample size studied was 248 with 122 males between 19-73 years and 126 females between 19-55 years of age. The number of subjects chosen was defined based on the minimum number required for partitioning of reference values. Age group was based on the NCCLS guideline which stated that reference individuals for the determination of a health-associated reference interval do not necessarily have to be young adults but may more closely resemble the patient population undergoing medical evaluation. All included volunteers were healthy and were selected based on a screening questionnaire from the NCCLS reference document. Choice of other anthropometric measurements was as per previous citations attempting a similar concept. Participants with the following conditions were excluded; diabetes mellitus, obesity, gout, hyperuricaemia, PCOS, dyslipidaemia, Cushing’s syndrome, acromegaly, insulinomas, thyroid disorders, rheumatoid arthritis, epilepsy and those with increased risk of clotting disorders.

Technical Information

Fasting plasma samples were collected with full aseptic precautions from all participants meeting inclusion criteria as per the screening questionnaire. Care was taken to avoid haemolytic samples both during collection and centrifugation procedures as presence of red cells is known to interfere with insulin assay results. Samples were analysed on the Abbott Architect Immunoassay analyser at the central reference laboratory in Mumbai of Metropolis Healthcare Pvt. Ltd. The Architect Insulin Assay is a closed Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of insulin in human serum or plasma. Assay standardisation, calibration, performance characteristics and quality control procedures were as per NCCLS guidelines and manufacturer’s assay kit insert. Assay sensitivity was ≤1.0 u IU/ml and cross-reactivity with proinsulin,
Table 1: Reference Limits for Fasting Insulin levels in a healthy adult Indian Cohort

<table>
<thead>
<tr>
<th>Analyte</th>
<th>No. of Observations (n)</th>
<th>Normality of distribution</th>
<th>Median (u IU/ml)</th>
<th>Mean (u IU/ml)</th>
<th>Minimum Value</th>
<th>Maximum Value</th>
<th>2.5% Limit (90% CI)</th>
<th>97.5% Limit (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma</td>
<td>Total = 248</td>
<td>Non-parametric</td>
<td>6.800</td>
<td>7.759</td>
<td>1.8</td>
<td>37.4</td>
<td>2.7225</td>
<td>16.905</td>
</tr>
<tr>
<td>Insulin</td>
<td>Males = 122, Females=126</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

C-peptide and glucagon was ≤ 0.1%, ≤ 0.001% and ≤ 0.001% respectively. Measurement range was between 1-300 u IU/ml and no detectable carryover (less than 0.5 u IU/ml) was observed even when samples containing 15,000 u IU/ml of insulin were assayed by the kit manufacturer.\(^{19}\) Laboratory inter and intraday assay co-efficient of variation (CV) were maintained at ≤ 5%.

Statistics

A non-parametric method of statistical analysis was used to determine the 2.5 and 97.5% (95% reference interval) limits with 90% confidence intervals.

Results

Data analysis was done with the help of SPSS Software ver 15 and Medcal ver 11. Table 1 summarises the derived reference limits in the study cohort. Since the analysis was non-parametric, no outliers were removed from the study dataset. A single reference limit range has been proposed for males and females as the observed means between both genders was not within statistically significant probability levels warranting separate reference limits.\(^{14}\)

Discussion

Elevated fasting insulin levels or hyperinsulinaemia has been mentioned in numerous citations for its varied association with a large number of metabolic disorders and is widely influenced by ethnicity. However, there are limited citations on diagnosing hyperinsulinaemia in normoglycaemic healthy Indians and also those striving to derive a reference range in this cohort.

Several ethnicities in native America and the Pacific region with increased prevalence of diabetes have demonstrated high mean levels of basal insulin relative to other populations.\(^{12}\) Likewise, Asian Indians are a group that is by nature more insulin resistant compared to other ethnic groups in South Asia.\(^{11,19-21}\) This has been widely documented in studies comprising of Indian cohorts from Malaysia and Singapore\(^{19,22}\) where hyperinsulinaemia along with dyslipidaemia and central obesity was a predominant risk factor contributing to the rise in prevalence of type 2 Diabetes Mellitus and cardiovascular disease in Indians over the last 3 decades.\(^{21}\) One of the foremost population studies on specific insulin response in native Asian Indians in 1998 confirmed that this population showed true elevated insulin responses despite a low BMI majorly because overall adiposity appeared to influence insulin secretion more than regional fat distribution in this ethnicity.\(^{23,24}\) Similar findings were reported from Asian Indians in the United Kingdom by Negi et al\(^{14}\) and also from studies in urban and rural South Indians.\(^{16}\)

Hyperinsulinaemia as a result of asian dietary habits

The consumption of refined grains in large carbohydrate meals is very common in Asian Indians, especially at dinner time. This permits hyperinsulinemia to occur and hence this group is more likely to develop metabolic syndrome in future.\(^{11,23}\)

Importance of diagnosing Hyperinsulinaemia in normoglycaemic adults and need for deriving ethnic reference ranges in Indians: Fasting insulin levels, one of the simplest indirect indices for diagnosing insulin resistance and basal hyperinsulinaemia in normoglycaemic adults constitutes an independent risk factor for metabolic deterioration to dysglycaemia in adults and helps identify healthy subjects at increased risk for diabetes.\(^{12,24}\) Earlier authors had suggested that the upper 25% in any general population are insulin resistant\(^{25}\) and evaluation of a future predictor of insulin resistance in normoglycaemic individuals has to be done by dividing them into either 4 or 5 quartiles of the existing reference limits. This has been reiterated in more recent publications\(^{7,25-28}\) which emphasise the need to derive ethnicity specific cut offs levels for insulin in order to accurately identify high risk normoglycaemics in the upper reference range quartiles and forms the basic hypothesis of our work.

In various studies of single ethnic groups, basal hyperinsulinaemia predicted the development of diabetes in Mexican Americans, Pacific Islanders and Pima Indians.\(^{12}\) Fasting insulin cut offs at the 75\(^{th}\) percentile were accurate at predicting insulin resistance in the normoglycaemic population in healthy Koreans.\(^{24}\) The San Antonio Heart Study showed that the presence of hyperinsulinaemia among healthy subjects predicted the subsequent appearance of diabetes, dyslipidaemia or hypertension over an 8-year follow up period.\(^{29}\) The Israel study of glucose intolerance, obesity and hypertension studying four
ethnic groups showed that the risk of developing dysglycaemia was almost twofold higher for the upper 5th quintile of fasting insulin than for the other four quintiles combined after adjusting for age. The RISC cohort which consisted of clinically healthy men and women across 14 European countries showed that fasting insulin could be an independent contributor to cardio metabolic risk and atherosclerosis in a healthy population. A limitation in these examples is that comparison between studies is cited to be unavailable due to absence of universal basal insulin cut-off levels, which is difficult to derive given the differential secretion patterns of this analyte across ethnic groups. Additionally, though hyperinsulinemia is mainly a predictor of insulin resistance and metabolic syndrome, it has also been implicated as a precursor in cardiovascular disease morbidity and mortality. Hirsutism and PCOS, sleep apnoea, non-alcoholic fatty liver disease and adiposity in individuals with normal or near normal blood glucose levels.

These observations led us to attempt derive ethnic reference ranges for plasma insulin in a normoglycaemic healthy Indian cohort as accurate laboratory results can be misleading if interpreted using inappropriate reference limits. Our work used the prospective unimodal approach to define reference limits for fasting plasma insulin in which a non-diseased reference sample group was selected using inappropriate reference limits. Our work laboratory results can be misleading if interpreted as accurate ethnic reference ranges for plasma insulin in a normoglycaemic healthy Indian cohort as accurate laboratory results can be misleading if interpreted using inappropriate reference limits. Our work

We noticed that results obtained in our analysis were consistent with similar reports published as early as 1967 in non-Indian cohorts. D. B. Grant showed that IRI levels in a group of 25 healthy adults ranged between 6-15 u U/ml with a single outlier result at 20 u U/ml. Though there were limitations with regards to the assay methodology of IRI and usage of serum instead of plasma, the range is still very close to that obtained using current assay platforms in our work.

In another publication where data from non-Hispanic-white, non-Hispanic-black and Hispanic (Mexican-American/other Hispanic) adolescents between 12 and 19 years of age were analysed, high fasting insulin levels were determined to be 16 IU/mL, the 95th percentile among normal-weight adolescents when computed by sex and ethnicity in the population under study. This is close to the upper limit of the derived reference range in our analysis which also advocates a cut off of 17 u IU/ml as the upper reference limit for hyperinsulinaemia in normoglycaemic Indian adults.

When compared with results in citations on Indians and Asian subjects, our derived lower reference limit of 2.72 u IU/ml was close to that seen in publications from 2009 onwards. However, it was much lower when compared to the lower reference limit of 7.2 and 9.36 u IU/ml stated in publications between 1994 and 1998. This could be because the method of analysis used in these publications was the older generation RIA, which had a higher lower limit of detection and greater cross reactivity with pro-insulin leading to estimation of higher insulin levels when compared to present day chemiluminescent platforms. The derived upper reference limit in our work was 16.9 u IU/ml when compared to upper limit cut offs of 10.8 and 11.8 m U/L that were seen in cited references. However, in all these citations, the total sample size was less than 120 for each gender, which is the minimum advocated for statistical derivation of reference limits. Details of fasting insulin reference levels from publications on Asians from 1998 to 2011 have been further elaborated below.

Snehalatha et al showed that fasting levels in normoglycaemic subjects for specific insulin was 9.36-10.80 uIU/ml. Another similar study studying the difference in plasma insulin responses in rural and urban South Indians showed that the range obtained in the rural group was 1.6-11.8 m U/L and that in the urban population was 7.2-26 m U/L. Higher insulin responses in the urban population were attributed to higher BMI, obesity, reduced physical activity and greater consumption of refined food.
Another work cited the baseline fasting plasma insulin levels in 10 normal weight and 10 low BMI Indian males from south India to range from 2.8-9.6 uU/ml and 0.7-10.9 uU/ml respectively.\(^3\)

The range of fasting insulin levels in men was 0.32-22.58 uU/ml and 3.18-20.82 uU/L in women in a study which included three major ethnic groups (Chinese, Malays, and Asian-Indians living in Singapore).\(^2\) However, the analysed dataset did not comprise of a pure normoglycaemic population and was performed on participants in previous population based cross sectional surveys carried out in Singapore from 1982-1998.

In a normoglycaemic group of Kuwaiti women, insulin reference limits were 3.9 – 10.1 mIU/L and 4.9-10.3 m IU/L when calculated using the mean and SD of women falling below the IR cut offs for HOMA-1 and 2 respectively in the study group.\(^38\)

Fasting insulin cut off points for the 1\(^{st}\), 2\(^{nd}\), 3\(^{rd}\) and 4\(^{th}\) quartiles in a healthy cohort of 2350 Koreans were ≤ 6.01, 6.02 to ≤ 7.29, 7.30 to ≤ 8.97 and ≥ 8.98 IU/ml respectively. Among subjects in the highest quartile for insulin levels, 16.4% subsequently developed MS in the whole cohort. The proportion of subjects who developed diabetes mellitus also significantly increased as the baseline fasting insulin level increased from first to fourth quartile. In the whole study cohort hyperinsulinaemia was an important predictor of future MS.\(^7\)

**Conclusion**

The clinical implication of our analysis would be to use the Indian ethnic reference ranges derived for fasting insulin in interpreting laboratory reports as currently used ranges have upper limits between 25-31 uIU/ml which is too high a cut off in normoglycaemics. A lower cut off of 17 uIU/ml or mU/L to define hyperinsulinaemia would be more appropriate in screening individuals who may be prone to a future risk of metabolic disorders. This is because though an elevated fasting insulin level has poor specificity for screening individuals who will go on to develop type 2 diabetes mellitus, it is still widely used in population based studies because of its strong association with metabolic syndrome and risk for later development of diabetes.\(^1\) Thus it is important to derive ethnicity specific cut off limits to define hyperinsulinaemia in normoglycaemic individuals and according to IFCC recommendations, the prospective unimodal reference interval approach used in this work is currently considered the gold standard for establishing reference ranges.\(^15,40\)

**Limitations**

The major limitation in this work is that the study cohort was mainly from the western region in India. Hence, though the derived reference limits may be used for interpretation of fasting insulin results in Indians from western India, further validation of these cut offs by transference from laboratories across different regions of the country would help improve robustness of these results.

**Future Research Directions**

This work may provide a basis for transference to other laboratories in India using similar instrumentation and method of analysis\(^35\) as a population specific validated fasting insulin reference range would help identify normoglycaemic Indians falling in the 4\(^{th}\) quartile cut offs who are known to have a ten times greater risk of developing insulin resistance, metabolic syndrome and other metabolic abnormalities compared to subjects in the lowest quartile. Lifestyle modifications may then be focused upon as the mainstay treatment in such individuals to prevent future metabolic syndrome and cardiovascular disorders.\(^7\)

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**References**