

Full Length Research Paper

Serum insulin-like growth factor-I adult reference values for an automated chemiluminescence immunoassay system

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Analysis of insulin-like growth factor I (IGF-I) in serum is fundamental in order to aid in the diagnosis of growth hormone (GH)-related disorders, as well as in the diagnosis and follow-up of other diseases. Considering the importance of IGF-I local normal range, the aim of this study was to determine reference values for IGF-I (Immulite 2000) in a Brazilian adult population from the city of Rio de Janeiro. This study included samples of blood taken from 484 healthy subjects (251 men, 233 women) aged 18 to 70. The subjects agreed with this study, approved by the ethical committee of the Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti, Rio de Janeiro, Brazil. The samples were analyzed by an automated immunochemiluminometric assay (ICMA). For data analysis, age and sex-specific figures were fitted after transformation of IGF-I values. In adulthood, a slow age-dependent decrease was seen. IGF-I in women were significantly higher than in men. The present study established age- and sex-specific IGF-I reference values, for the healthy Brazilian adult population from the city of Rio de Janeiro, determined with the currently most widely IGF-I assay used in Brazil (Immulite 2000).

Key words: Insulin-like growth factor I, chemiluminescence assay, reference range.

INTRODUCTION

Serum insulin-like growth factor I (IGF-I) assays have been found to be useful as a screening test for the presence of growth hormone (GH) deficiency and to monitor the response to GH treatment in adults (Drake et al., 2001). IGF-I has also been found very important in establishing the diagnosis of acromegaly (Cordero and

Barkan, 2008) and in monitoring the response to treatment of acromegaly (Roemmler et al., 2009). In addition to these applications in GH-related disorders, recent studies have focused on a possible relation between IGF-I levels and the risk for certain malignancies as well as on a prognostic significance of IGF-I levels in patients with malignant diseases (Janssen et al., 2004). Friedrich et al. (2011) reported a relation between IGF-I and all-cause mortality in male primary care patients. These developments indicate that analysis and interpretation of IGF-I levels shall comprise an important component which is widely used in the diagnosis and follow-up of many disorders (Ben-Shlomo and Melmed, 2001). It is thus

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Abbreviations: IGF-I, Insulin-like growth factor I; GH, growth hormone; ICMA, immunochemiluminometric assay.

Table 1. Age and sex distribution of the studied population .

Subject	Age (year)							All subject
	18-20	21-25	26-30	31-40	41-50	51-60	61-70	
Men	30	38	37	36	37	40	33	251
Women	31	37	31	34	34	32	34	233
Total	61	75	68	70	71	72	67	484

imperative that uniform interpretations of IGF-I levels be achieved by improving the standardization and inter-laboratory comparability of IGF-I analysis procedures (Quarby et al., 1998). This includes the establishment of normative data in representative populations by valid immunoassays and by considering non-GH-related factors known to influence IGF-I concentrations like age and sex (Ranke et al., 2001, 2003; Barkan et al., 2007). Despite of the well known necessity of IGF-I local reference range (Clemmons, 2007), to our knowledge, there is only one study evaluating IGF-1 reference range in the Brazilian population (Rosario, 2010), with people from the metropolitan region of Belo Horizonte, Minas Gerais State.

The determination of the serum levels of IGF-I has been performed by some methods, as the immunoradiometric assay (IRMA) and the immunochemiluminometric assay (ICMA). ICMA has been widely used in the world in determination of the values of the IGF-I and other biological markers in the human serum (Brabant et al., 2003; Friedrich et al., 2011). The Immulite 2000 assay is an ICMA commercial kit (IMMULITE/ IMMULITE IGF-1, 2006). Nowadays, the ICMA (Immulite 2000), chosen in this study is the assay currently used at various Neuroendocrinology centers and clinical analysis laboratories in Rio de Janeiro city, as in the most part of Brazil (Rosario, 2010). In the case of the assay tested in this present study, the reference values are derived from a German population (Elmlinger, 2004).

To improve IGF-I measurement, the calibration of assays against a new preparation of recombinant human IGF-I (rhIGF-I) produced by the WHO First International Standard (WHO IS 02/254) has been recommended (Burns et al., 2009; Clemmons, 2011).

Considering the necessity to determine local normal ranges and because Brazil is an almost continental country, the aim of this study was to determine reference values for IGF-I (Immulite 2000) in a healthy Brazilian adult population from the city of Rio de Janeiro, Rio de Janeiro State.

MATERIALS AND METHODS

Ethical approach

Subjects were either selected from blood-bank donors or general population. Informed consent was obtained from all the subjects. This study protocol had been approved by the ethical committee of

the Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti, Rio de Janeiro, RJ, Brazil.

Excluded criteria

Subjects that has taken any medications that interfere in IGF-I analyses (contraceptive drugs, estrogens, corticosteroids) were not considered to be in this investigation. Also, subjects with renal diseases, liver diseases, malignant disorders, diabetes mellitus and diseases of the pituitary gland, were excluded from this investigation. These information were obtained directly by medical history in a private interview.

Subjects

IGF-I was determined in samples from 484 healthy subjects (251 men, 233 women) aged from 18 to 70 (Table 1), selected from blood-bank donors or general population of the Rio de Janeiro city, Brazil. Seven groups divided according to age were pre-defined, with a minimum of 60 subjects per group (Ranke et al., 2003). Since the need of different reference values for men and women is not clear (Brabant and Wallaschofski, 2007), each group was divided into two subgroups (at least 30 men and 30 women). Each participant selected was allocated according to gender and age until the predefined number of subjects per subgroup was completed.

Serum samples

The subjects were from the Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti, Rio de Janeiro, RJ, Brazil. Samples of 10 ml of venous whole blood were collected, in fasting state, between 8 and 10 h, in plastic gel barrier tubes not containing any anticoagulant. Furthermore, these samples were routinely sent to the Laboratório Helion Póvoa, Rio de Janeiro, Brazil to be processed with a maximum time interval of 90 min between sampling and freezing. Serum was separated and stored frozen at -20°C up to the analysis. Samples were assayed within 2 to 3 months after sampling. Grossly hemolytic, lipemic or icteric samples were excluded. Prior to the IGF-I determination, the samples were allowed to reach room temperature.

Analysis of IGF-I

Serum IGF-I concentrations were determined by an automated, two-site immunochemiluminescent assay system (Immulite 2000/ Diagnostic Products Corp., Los Angeles, CA). Immulite 2000 IGF-I is a solid-phase, enzyme-labeled chemiluminescent immunometric assay with an analytical sensitivity of 25 µg/L, intra- and interassay coefficient of variation < 8%, standards calibrated against the WHO is 87/518 preparation (Bristow, 1990), and the reference values provided by the manufacturer (Elmlinger et al., 2004). The steps of

Table 2. Serum IGF-I (ng/ml) reference ranges for Immulite 2000 at ages 18 to 70.

Parameter	-2SD	-1SD	Mean	+1SD	+2SD
ICMA – Men age in years					
18 - 20	177.68	230.44	298.87	387.61	502.70
21 - 25	149.61	194.03	251.64	326.36	423.27
26 - 30	96.06	140.47	205.41	300.37	439.22
31 - 40	105.95	137.41	178.22	231.13	299.77
41 - 50	70.18	99.58	141.32	200.54	284.58
51 - 60	82.02	106.38	137.96	178.93	232.06
61 - 70	71.74	95.87	128.12	171.23	228.83
ICMA – Women age in years					
18- 20	178.39	238.41	318.62	425.81	569.07
21 - 25	173.12	226.78	297.08	389.16	509.79
26 - 30	100.99	143.31	203.36	288.59	409.53
31 - 40	84.02	126.60	190.76	287.44	433.11
41 - 50	81.45	112.17	154.47	212.72	292.95
51 - 60	54.65	84.86	131.76	204.59	317.67
61 – 70	54.93	77.94	110.61	156.96	222.74

the protocol to the assays have followed manufacturer indications (IMMULITE/IMMULITE IGF-1, 2006). Briefly, this system uses beads coated with monoclonal murine anti-IGF-I for capture and alkaline phosphatase (bovine calf intestine) conjugated to polyclonal rabbit anti-IGF-I in buffer for detection. The antibody is highly specific for IGF-I. Measurement of IGF-I in serum is complicated by the presence of acid-labile components and binding proteins. Acid treatment is necessary to release IGF-I to ensure accurate quantitation. Subject samples are acidified to separate IGF-I from IGF binding proteins. This displacement method was found to be very accurate. Calibration range is up to 1600 ng. Analytical sensitivity is 20 ng/ml.

To ensure proper instrument performance and allow the comparison between runs, IGF-I control samples (three levels) were analyzed in each batch of reference samples. All runs with one or more controls out of given ranges (mean \pm 2 SD) were recalibrated until all controls were in range.

Statistical analysis

In order to find a scale with as constant variance as possible for the observations, different transformations of the IGF-I values were compared (BOX-COX (p-value <0.001), square root (p-value < 0.001), log (p-value 0.27)). The analysis of the data recommended logarithmic transformation of IGF-I data to establish normal ranges (Armitage et al., 2002). The reference range was defined using logarithmic transformation of the values obtained, calculation of the mean \pm 1 and \pm 2 SD of these values, and exponentiation to obtain the limits corresponding to the original scale (Brabant et al., 2003; Elmlinger et al., 2004; Massart and Poirier, 2006). Then, we decided to fit age and sex-specific evaluations following data transformation.

Gender contribution was tested in the final analysis. Potential interaction between age and gender was tested using Slope test for men and women data at three different cut levels: 30, 40, and 50 years. R Program - version 2.9.0 (R Development Core Team,

2009) was used for the statistical analyses.

RESULTS

The transformation that best converted these IGF-I data to normal distribution was the power 0.27. Mean value, SD and reference limits was calculated on the IGF-I^{0.27} scale and then transformed back (Table 2).

As shown in Figure 1, the intersection between men and women curves represented interaction between age and gender, with an inversion between men and women results around 50 years of age. Women had statistically (p < 0.05) higher values than men up to 50 years of age. After 50 years of age, women had slightly lower values.

The mean and standard deviation (SD) of the IGF-I values were dependent on age. After puberty peak, serum IGF-I decreased rapidly to approximately 25 years of age. From there, a slow decrease began, persisting up to at least 70 years of age (Figure 2 and Table 2).

When Slope Test was used incorporating an interaction between gender and age, the data showed a statistically significant difference in levels between men and women. The p-value for both genders was 0.0181. Due to this significant effect of gender and of different standard deviation sizes, we decided to fit two separate figures, one for each gender as shown in Figure 2a, Figure 2b. Table 2 shows read values from Figure 2a, b for ages 18 year up to 70 for men and women. The difference between fitted gender-specific means was greatest between ages 18 and 25, when women had fitted means 19 to 45 ng/ml higher. Between ages 25 to 50 women

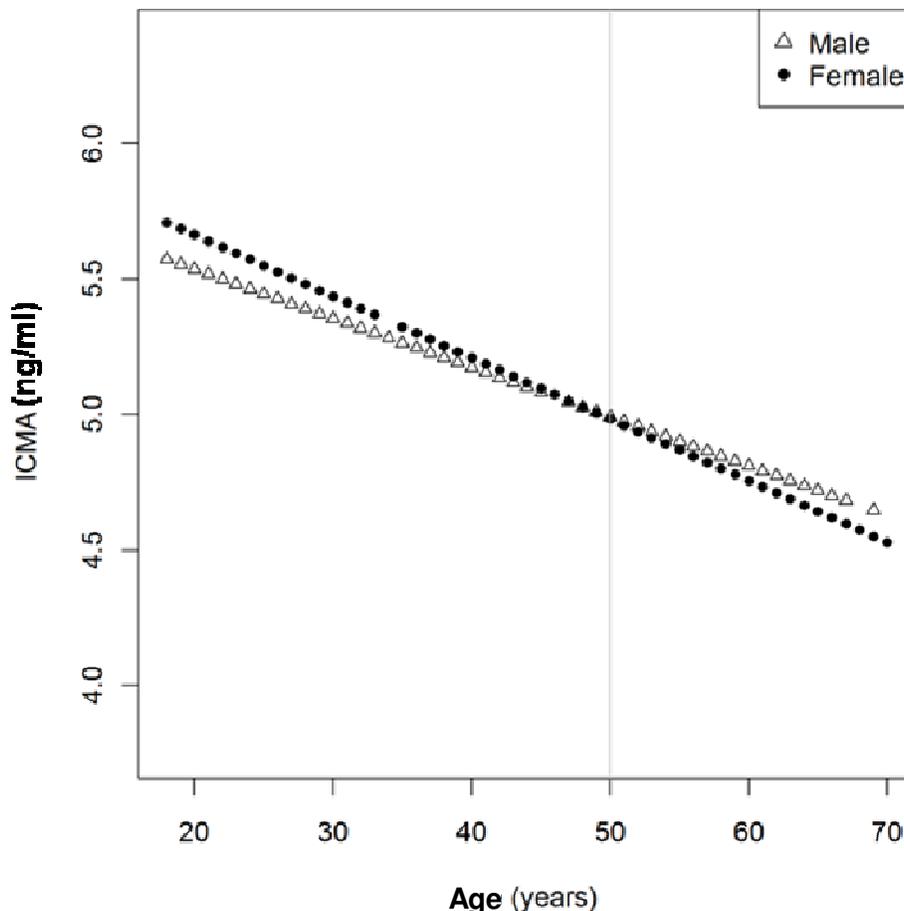


Figure 1. Slope test for men and women data with cut level at 50 years; slope test between genders – ICMA (p-value: 0.0181).

had fitted means around 12 ng/ml higher. At ages above 50, men had 6 to 17 ng/ml higher mean values. The smallest between-gender difference was 2 ng/ml. This was seen between ages 26 and 30. Moreover, the reference range was a bit broader for women compared to men, for a given age (Table 2). The distribution of observed serum IGF-I values closely followed what was expected from the log normal distribution (Figure 2).

DISCUSSION

IGF-I, while being GH-dependent, is also regulated by multiple other factors including nutrient intake, nutritional status, thyroxine, estrogen, cortisol and genetic background. In addition, the individual levels of IGF-binding proteins (IGFBP) can have major influences on IGF-I levels (Clemmons, 2007). Considering these knowledge, local reference range should be established (Clemmons, 2007). Brazil is an almost continental country and the only one studying evaluating IGF-1 reference range in the Brazilian population, done with people from the metropolitan region of Belo Horizonte, Minas Gerais (Rosario,

2010).

The aim of this investigation was to generate data for IGF-I (Immulite 2000) in a clinically well-characterized healthy Brazilian adult population from the city of Rio de Janeiro, under normal routine situation. In this present study, reference values for IGF-I was established based on a large number of healthy subjects between ages 18 and 70. The results of this study were substantially different from the results of the another one performed with subjects from the metropolitan region of Belo Horizonte, Brazil (Rosario, 2010), supporting the necessity of establishment of local reference range. In this study of Rosario (2010), no difference in IGF-I levels was observed between genders.

The need for assay-specific IGF-I reference values has been well established. The Immulite 2000 assay used in this study is yet standardized against the WHO IS 87/518. Otherwise, this method is widely used at various Neuroendocrinology centers and clinical analysis laboratories in Brazil (Rosario, 2010) and in others countries (Elmlinger, 2004). This fact has stimulated the realization of our investigation with this commercial assay. In the future, this automated assay probably will

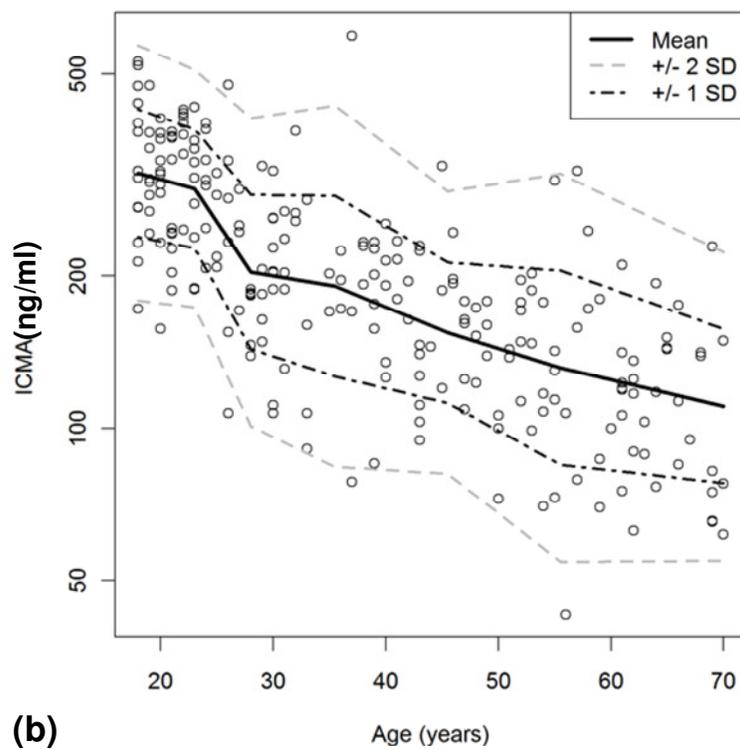
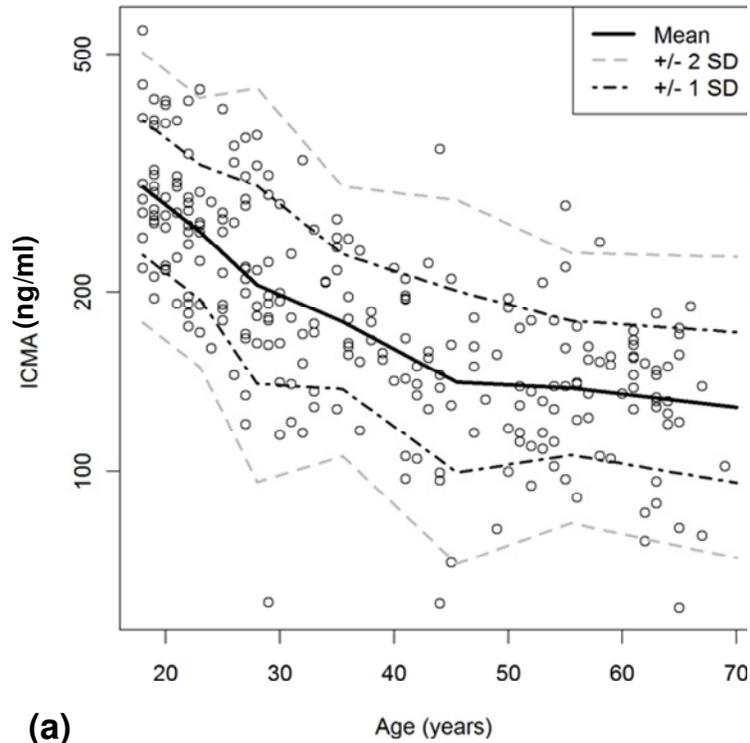


Figure 2. Serum IGF-I data as function of age. The figure presents the individual values of the 484 healthy subjects (251 men and 233 women) as well as the curves for the fitted mean, ± 1 SD and ± 2 SD functions. Scattergrams are given for men (Figure a) and women (Figure b) separately, after logarithmic transformation. (a) - IGF-I data as function of age for men, (b) - IGF-I data as function of age for women.

be calibrated against the WHO IS 02/254 and reference values will be determined using this recalibrated assay.

The major variability of reference laboratory assays for establishing useful normative data seems to be the number of subjects collected (Clemmons, 2007). If adequate numbers of subjects are collected, then normal ranges should be reasonably comparable across various assays methodologies. In a study by Ranke et al. (2003) using 427 normal adults, in which a standard radio-immunoassay using excess IGF-II was compared to an IRMA and an ICMA assay, there was excellent comparability. The correlation coefficients varied between 0.79 and 0.87 for the 427 normal adults. Thus, for establishment of normal ranges, it is likely that techniques that use high affinity, high specificity antibodies and some method for eliminating binding protein interference will give reproducible comparisons for values within the normal range, if adequate numbers of subjects are collected.

By having a large number of healthy subjects involved in this work to establish reference ranges for an analyte, it was possible to eliminate systematic analytical errors before the reference samples were analyzed. In our study, our findings have involved 484 subjects. The size of this investigation is close to the study reported by Ranke et al. (2003).

Logarithmic transformation of IGF-I data approach makes the reference values well suited for monitoring of IGF-I levels by Immulite 2000/DPC system. Moreover, the authors recommend logarithmic transformation of normative data to establish normal ranges, since the normative dataset follow a lognormal distribution (Armitage et al., 2002.).

IGF-I values rise from birth until puberty and then decline with advancing age (Bagg et al., 2006; Brooke and Drake, 2007). Over the duration of the life span these changes are quite extensive, that is, from birth to puberty the values can increase by a mean of 5-fold and, conversely, between puberty and age 80 they can decline 3.5-fold. Therefore, it is necessary to establish age adjusted ranges (Brabant et al., 2003; Kwan and Hartman, 2007). During childhood, particularly in the years surrounding puberty, the age intervals need to be relatively narrow. No more than 3 to 4 years included in each group. In contrast, after age 30, the rate of decline of IGF-I was lower, so that grouping by decade was reasonable.

In adult subjects, our results show a steady decrease of serum IGF-I levels with age, which has already been demonstrated by other authors (Strasburger et al., 2001; Friedrich et al., 2008; Jones and Honour, 2006; Li et al., 2005; Massart and Poirier, 2006; Barkan, 2004). In addition to this, wellknown age-dependent IGF-I decrease, this study indicated a gender difference in the adult reference ranges with adult women until age 50, having slightly, but significantly higher IGF-I values than men. The gender difference was inverted after 50 years of age with men having slightly higher IGF-I values than

women. Moreover, the reference range for women were generally a bit broader compared to that of men. A possible explanation for these gender differences may be the effect of estrogen in women before menopause, which is well known to influence serum IGF-I levels (Anderson et al., 2001; Jernström et al. 2001).

Another finding of this study was that the values of some few reference samples differed significantly from the total mean. This result obviously reflects the influence of several factors which could affect IGF-I levels, as differences between subjects with respect to genetic background or nutritional status, pre-analytical influences like sample collection and storage, as well as analytical interferences (Chestnut and Quarby, 2002; Yuen et al., 2004). The finding underlines the fact that selection of a representative reference population is a delicate task and that a big sample size reduces the risk of a non-desirable impact from a single or few subjects.

In conclusion, this present study has established age- and sex-specific serum IGF-I reference values for the Brazilian adult population from Rio de Janeiro city, based on a large number of healthy subjects, and with the currently most widely IGF-I assay used in Brazil (Immulite 2000/DPC). The reference values may be used in different laboratories, since systematic difference between systems is low using the same calibrators and establishing an efficient intra- and inter-laboratory control procedure.

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