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Reference values of IGF1, IGFBP3 and IGF1/IGFBP3 ratio in adult population in the Czech Republic

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ABSTRACT

Background: IGF1 is responsible for regulation of growth, metabolism and differentiation of human cells. IGFBP3 is the most abundant of the carrier proteins for IGF1 in the blood. IGF1/IGFBP3 molar ratio is an indicator of IGF1 bioavailability. We decided to create a file of reference ranges of IGF1, IGFBP3 and IGF1/IGFBPP3 ratio for the adult Czech population across the age spectrum.

Methods: We selected a group of 1022 subjects, 467 males and 555 females (ages 20–98 years), from several regions in the Czech Republic. The group consisted of blood donors and patients undergoing regular preventive examinations. Serum levels of IGF1 and IGFBP3 were measured using the following radioimmunoassay kits: IRMA IGF1 (Immunotech, Marseille, France) and IRMA IGFBP3 (Immunotech, Prague, Czech Republic). The IGF1/IGFBP3 ratio was also calculated. The following groups of patients were excluded: patients with diabetes, high blood glucose, high insulin levels, post-surgery patients, polymorbid patients, and subjects with oncological diseases. Subjects were divided into seven age-groups. Changes in the levels of observed analytes in each decade across the age spectrum were evaluated. All statistical analyses were performed by SAS 9.3 (Statistical Analysis Software release 9.3; SAS Institute Inc., Cary, NC, USA).

Results: All three parameters IGF1, IGFBP3 and IGF1/IGFBP3 decreased in parallel with decrease in age: $p < 0.0001$, $r = -0.64$, -0.35 and -0.54, respectively. The dynamics of the decline was different between males and females.

Linear regression models with age as independent variable fitted by gender are displayed in Fig. 1. Non-parametric reference interval curves (medians and 2.5th–97.5th percentiles) for IGF1, IGFBP3 and IGF1/IGFBP3 ratio as function of age by gender are displayed in Fig. 2(a,b,c). All medians and 2.5th–97.5th percentiles were plotted by cubic spline.

For males, linear regression models were as follows: IGF1 = 291.34619 − 2.41211 × age, IGFBP3 = 2931.62778 − 6.11659 × age, IGF1/IGFBP3 = 0.02897 − 0.00021213 × age. For females, we plotted the following: IGF1 = 241.67406 − 1.98466 × age, IGFBP3 = 3688.60561 − 16.39560 × age, IGF1/IGFBP3 = 0.02029 − 0.00013233 × age.

IGF1 was statistically significantly higher in males with $p < 0.0001$ (Wilcoxon test) but decreased faster ($p = 0.0121$). IGFBP3 was statistically significantly higher in females with $p = 0.0004$ (Wilcoxon test) but decreased faster ($p < 0.0001$). IGF1/IGFBP3 was statistically significantly higher in males with $p < 0.0001$ (Wilcoxon test) but decreased faster ($p < 0.0001$).

Conclusion: Authors recommend using of a linear regression model based reference ranges for IGF1, IGFBP3 and IGF1/IGFBP3 ratio and using different reference ranges for genders.

1. Introduction

IGF1 is responsible for regulation of growth, metabolism, and differentiation of human cells. IGFBP3 is the most abundant of the carrier proteins for IGF1in the blood [1]. Levels of both IGF1 and IGFBP3 decrease with age. Serum levels of IGF1 and IGFBP3 are commonly used for monitoring growth hormone (GH) production in children and adults with growth disorders [2,3]. Several studies have also related IGF1 serum levels with risk for certain malignant tumors [4] as well as for various other clinical disorders. [5–8].

We investigated the relationship between IGF1 and IGFBP3 serum levels and at the same time looked at the potential of the IGF1/IGFBP3

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molar ratio as an indicator of IGF1 bioavailability [7]. Finally, we created a file of reference ranges of IGF1, IGFBP3 and IGF1/IGFBPP3 ratio for the adult population across the age spectrum.

2. Methods

2.1. Group of patients

We examined a group of 1022 subjects (467 males and 555 females) between the ages of 20 to 98 years old. The group consisted of blood donors and patients undergoing regular preventive examinations. The following groups of patients were excluded from the study: patients with diabetes, high blood glucose, or high insulin levels, patients after surgery, polymorbid patients, people with oncological diseases, with renal diseases, liver diseases, diabetes mellitus, diseases of the pituitary gland and subjects using any medications that interfere in IGF1 analyses (contraceptive drugs, estrogens, corticosteroids).

We divided the adult subjects into seven groups according to age-groups: 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, and 80 + years, and sex. We evaluated the changes in the levels of observed analytes and the changes in IGF1/IGFBP3 ratio in each decade across the age spectrum.

2.2. Serum samples

Samples of venous blood were collected using the VACUETTE blood collection system (Greiner Bio-one Company, Kremsmünster, Austria). The separation of red blood cells was done till 2 h after collection. Blood was centrifuged for 10 min at 1700 g. Serum samples were immediately frozen at –80°C. Samples were thawed only once, just prior to analyses.

2.3. Sample analysis

We measured IGF1 serum levels using radioimmunoassay kit IRMA IGF1 (Immunotech, Marseille, France), with interassay CV of 6.8% at a concentration of 25.0 ng/mL, and analytical sensitivity of 2.0 ng/mL. The calibrators in the Immunotech IGF1 assay are calibrated against the international reference standard, WHO 87/518.

IGFBP3 serum levels were measured using radioimmunoassay kit IRMA IGFBP3 (Immunotech, Prague, Czech Republic), with interassay CVs of 6.23% at a concentration of 61.5 ng/mL, and analytical sensitivity of 0.27 ng/mL.

We calculated the IGF1/IGFBP3 molar ratio based on the following: 1 ng/ml IGF1 = 0.130 nmol IGF1 and 1 ng/ml IGFBP3 = 0.036 nmol IGFBP3 [9].

2.4. Statistical analysis

Quantitative variables are reported as medians, mean, Q1, Q3, 2.5th–97.5th percentiles, minimum and maximum. Age-interval-specific reference intervals for all parameters were calculated as 2.5th–97.5th percentiles. Different statistical approaches were tested.

The calculated 2.5th–97.5th percentiles were plotted by cubic splines, and gender-specific curves were generated.

Different statistical approaches were tested. First, a linear regression model with age as independent variable fitted by gender was calculated [10]. Second, a non-paramateric model of reference interval curves (medians and 2.5th–97.5th percentiles) for IGF1, IGFBP3, IGF1/IGFBP3 ratio as function of age by gender was calculated [11]. All medians and 2.5th–97.5th percentiles were plotted by cubic splines.

Linear regression models with age as independent variable fitted by gender appeared to be the more robust model with similar results as non-parametric approach and the parametric method described by Wright and Royston, where basic transformations of the data and multiple regression techniques are combined.

All statistical analyses were carried out with SAS 9.3 (Statistical Analysis Software release 9.2; SAS Institute Inc., Cary, NC, USA).

3. Results

We performed the calculation of reference values of IGF1, IGFBP3 and IGF1/IGFBP3 ratio for men (Table 1), women (Table 2), separately and for the whole population (Table 3). Then we focused on the analysis of differences between male and female ranges.

All three parameters IGF1, IGFBP3 and IGF1/IGFBP3 decreased with age with $p < 0.0001$, $r = -0.64$, -0.35 and -0.54, respectively. The dynamics of the decline was different between males and females.

Table 1
IGF1, IGFBP3 and IGF1/IGFBP3 ratio in males.

Age category	N	Mean	Minimum	2.5th percentile	Lower quartile	Median	Upper quartile	97.5th percentile	Maximum
IGF1 in males									
20–29	52	**242.37**	84.43	100.80	202.10	**239.70**	282.60	400.00	403.10
30–39	62	**202.65**	100.40	101.90	164.60	**192.15**	242.70	341.21	341.50
40–49	67	**167.66**	75.25	103.70	140.00	**163.60**	188.40	264.90	268.30
50–59	95	**160.82**	46.60	77.14	132.60	**155.83**	183.20	296.60	339.30
60–69	75	**143.55**	37.42	39.30	105.60	**145.40**	182.70	241.20	264.60
70–79	66	**110.15**	19.70	33.85	67.70	**101.50**	147.70	219.60	290.10
80+	50	**96.18**	11.20	39.50	65.80	**99.30**	126.00	150.00	185.30
IGFBP3 in males									
20–29	52	**2879.4**	1887.5	1922.0	2510.5	**2838.8**	3096.5	3959.0	4621.0
30–39	62	**2688.7**	1238.0	1927.0	2440.0	**2651.0**	2879.5	3967.5	4142.0
40–49	67	**2590.2**	1711.0	1966.5	2340.0	**2585.5**	2769.5	3486.0	3545.0
50–59	95	**2547.7**	1816.0	1863.0	2288.5	**2515.0**	2723.5	3514.0	3580.5
60–69	75	**2520.1**	1204.5	1572.0	2097.5	**2474.0**	3015.0	3423.5	3802.0
70–79	66	**2492.9**	1063.5	1631.5	1959.0	**2433.8**	2867.5	3812.0	3828.5
80+	50	**2499.3**	1401.0	1425.0	1893.0	**2356.3**	3001.0	4570.5	4570.5
IGF1/IGFBP3 molar ratio in males									
20–29	52	**0.02418**	0.00877	0.01389	0.01910	**0.02378**	0.02939	0.03546	0.03992
30–39	62	**0.02147**	0.01155	0.01508	0.01730	**0.02115**	0.02414	0.03407	0.04077
40–49	67	**0.01815**	0.00907	0.00912	0.01591	**0.01725**	0.02110	0.02778	0.02963
50–59	95	**0.01785**	0.01019	0.01108	0.01426	**0.01751**	0.02050	0.02645	0.02908
60–69	75	**0.01671**	0.00573	0.00673	0.01308	**0.01626**	0.01974	0.02973	0.03018
70–79	66	**0.01296**	0.00376	0.00416	0.00691	**0.01219**	0.01689	0.02568	0.02935
80+	50	**0.01133**	0.00242	0.00388	0.00775	**0.01098**	0.01355	0.02157	0.02603