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Original Research Article

Verification of Roche reference ranges for serum prolactin in children, adolescents, adults, and the elderly

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ABSTRACT

Background: Reference intervals (RIs) for prolactin are of high clinical importance for diagnosis, treatment, and prognosis of hypothalamic-pituitary disorders. The aim of this study was to verify the Roche reference ranges for serum prolactin in children, adolescents, adults, and the elderly.
Materials and Methods: An indirect method based on currently laboratory data was used on the reference ranges. Nine thousand one hundred and thirteen prolactin results were included. Pregnancy, lactation, exercise and reported pathologies were ruled out and only samples were collected between 8AM and 12PM were used. Reference ranges with a confidence interval of 95% (95% CI) were estimated.
Results: Compared to the manufacturer's reference values and for the corresponding age group, the median values obtained in our study were 10 – 60% higher depending on the age and sex of the patients. Elevated levels of serum prolactin were observed in the neonatal period and values decrease until median values near 200 mUI/L in childhood. During the children's period, no gender differences were observed for prolactin level. The gender difference in prolactin levels became significant from pre-adolescence until the age of 60. Prolactin levels increased significantly ($p<0.001$) between children and young adults, followed by a gradual and continuous decrease until young senior age.
Conclusion: The prolactin reference values proposed by the manufacturer in the data sheet appeared unsuitable. Laboratories should review reference ranges, and a partitioning with sex and different age groups may be appropriate.

1. Introduction

Prolactin (PRL) is a polypeptide hormone produced by lactotropic cells in the anterior pituitary gland.[1] During pregnancy, a woman's body starts to produce higher than normal levels of prolactin. This hormonal increase causes the mammary glands to begin preparation for milk production. Its elevation in men and in non-pregnant or non-breastfeeding women is considered pathological and may indicate a type of tumor of the pituitary gland, known as a prolactinoma.[2] The mature hormone is composed of 199 amino acids with a molar mass of 23 kDa. It is present in the circulating blood in several molecular forms: the glycosylated monomeric prolactin (23 – 25 kDa), the big prolactin (50 – 60 kDa), the structure of which is not clearly established, and the big big prolactin or macroprolactin (150 – 170 kDa), which is most often made up of a prolactin molecule linked to an IgG.[3] Macroprolactin and big prolactin could be recognised, at least in part, by current immunoassays and they can be responsible for false hyperprolactinemia,[4] but Fahie-Wilson and al.[5] showed that the Roche Elecsys Prolactin II assay had a lower reactivity with macroprolactin. In 2019, De Sousa SMC et al[6] demonstrated that serum prolactin was overestimated with the Roche method compared to other

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platform, and it was recommended that laboratories review the Roche reference. In our laboratory, discrepancies were observed between hyperprolactinemia measured with the Roche Elecsys Prolactin II reagent and normal results with a radioimmunoassay method (with and without polyethylene glycol precipitation). The cause of prolactin overestimation was unclear. The aim of our retrospective study was to verify that the reference values provided by the manufacturer were transferable to our own patient population, and to determine different reference ranges for serum prolactin in children, adolescents, adults, and the elderly.

2. Materials and Methods

2.1. Data collection

An indirect method based on currently laboratory data was used on the reference ranges.[7] All prolactin results reported between March 2017 and December 2019 were collected, and statistical analysis was performed. Retrospective data were used in accordance with the ethical standards of EU regulation 2016/676 on the protection of natural persons and the processing of personal data. All data were then anonymized. The Inovie-Labosud database is registered with the French National Commission on Informatics and Liberty, record no. 2073511v0.

2.2. Clinical exclusion criteria

Pregnancy, lactation, exercise, hormonal therapy for infertility and reported pathologies were ruled out by laboratory investigation to minimize their possible effect on prolactin level. Considering the diurnal cycle of prolactin secretion,[8] all samples were collected between 8AM and 12PM. To ensure that the patient did not do any kind of exercise, a rest period of 15 minutes was required before giving the blood sample.

2.3. Detecting and eliminating outliers

A major part of statistical analysis is the identification of outliers in the data. The process illustrated by John Tukey[9] was used to find outliers. The rules of the method are as follows:

1. The first quartile Q1 is the value ≥ 1/4 of the data, the second quartile Q2 or the median is the value ≥ 1/2 of the data, and the third quartile Q3 is the value ≥ 3/4 of the data.
2. The IQR (Inter Quartile Range) is the distance between the lower (Q1) and upper (Q3) quartiles.
3. Tukey outliers are data points that lie outside the following range [Q1-1.5 IQR, Q3+1.5IQR].

2.4. Establishment reference ranges

The reference ranges with a 95% confidence interval (95% CI) were estimated according to the recommendation of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM).[10] The expected values were the central 95th percentile based on nonparametric estimates defined by the 2.5th and 97.5th percentiles as the lower and upper reference limits, respectively.

2.5. Study population and partitioning

The study population consisted of 9113 subjects aged between 1 month and 95 years. Age and gender partitioning have been estimated based on graphic visualization of our data. In some cases, partitioning led to creation of groups with less than 120 observations. Each sub distribution is given in Table 1.

3. Method, instrument, and quality controls

Prolactin was measured using the PRL II assays on the Cobas ® e 801 analytical unit (Roche Diagnostics, Basel, Switzerland). The measuring range of the PRL II assay extends from 2 to 10,000 mIU/L. Between run imprecision determined with the PRL II assay and Elecsys PreciControl Universal (PCU I and II) showed a CV of 4.5% and 4.1% respectively. External quality assessments provided by Probioqual ® (Lyon, France) were regularly evaluated for prolactin. Analytical bias was estimated using externalised internal quality control against the peer group. During the study period, the mean bias was 0.4% with a standard deviation of 2.2%.

3.1. Statistical analysis

A shapiro-wilk test showed a non-parametric distribution of results and the comparison between groups was tested using a Mann-Whitney test (differences between populations were estimated as significant at $p<0.05$).

4. Results

Reference ranges, age and gender groups are summarized in Table 2. Prolactin results for all age and sex stratifications showed non-parametric distributions ($p<0.001$). Reference ranges (95% intervals) were studied from the age of two years onwards according to age, sex, and sexual maturation (physical changes in females usually start after age 12 and around age 13 for males).

4.1. Age and sex dependent changes in serum prolactin.

The Figure 1 showed the median serum prolactin levels using the PRL II assays from newborn to senior life. After elevated levels of serum prolactin are observed during the first year of life, values decrease significantly

Table 1: Partitioning of sampling groups

Sampling groups	Females age	Males ages
1^{st} baby group	< 1 year (both sexes)	
2^{nd} baby group	> 1 year (both sexes)	
Children	2 – 9 years	2 – 10 years
Pre-adolescents	10 – 12 years	11 – 13 years
Adolescents	13 – 16 years	14 – 16 years
Young adults	17 – 19 years	17 – 19 years
1^{st} Adults group	20 – 30 years	20 – 30 years
2^{nd} Adults group	31 – 40 years	31 – 40 years
1^{st} Middle-aged adults' group	41 – 50 years	41 – 50 years
2^{nd} Middle-aged adults' group	51 – 60 years	51 – 60 years
Young seniors	61 – 70 years	61 – 70 years
Seniors	> 70 years	> 70 years

Table 2: Descriptive statistics for serum prolactin in children, adolescents, adults, and seniors

Age group	N	Lower referencelimit (95% CI)	Upper referencelimit (95% CI)	Median (95% CI)
Infants (both sexes) (results are expressed in mUI/L				
<1 y	86	120.8 (12.0–131.4)	1542.0 (1441–1614)	370.6 (331.1–464.2)
1 y	89	100.0 (12.0–120.8)	815.0 (745.2–1299.0)	355.0 (319.0–435.4)
Female over one year old (results are expressed in mUI/L				
2–9 y	138	82.8 (68,9–97.8)	453.0 (397.0–529.3)	194.8 (177.0–208.2)
10–12 y	95	121.8 (104.1–133.5)	578.7 (520.5–653.0)	273.9 (249.4–303.9)
13–16 y	655	92.4 (53.3–112.3)	762.1 (724.5–781.4)	352.6 (343.2–371.1)
17–19 y	853	70.9 (57.5–90.7)	864,4 (834.6–888.1)	397.0 (386.4–416.2)
20–30 y	318	122.0 (91.4–130.65)	853.3 (782.0–892.3)	356.5 (340.7–371.4)
31–40 y	341	102.4 (62.1–109.3)	771.9 (709.9–816.2)	325.4 (301.5–357.1)
41–50 y	161	128.9 (102.6–137.4)	716.2 (615.4–746.3)	292.9 (269.7–318.0)
51–60 y	1353	70.5 (47.1–77.9)	712.7 (681.9–727.3)	272.4 (262.1–281.8)
61–70 y	451	44.5 (28.1–62.2)	518.2 (474.8–547.0)	227.9 (211.8-237.3)
>70 y	209	56.8 (20.7–78.3)	511.8 (474.3–528.3)	250.3 (237.3–267.2)
Male over one year old (results are expressed in mUI/L				
2–10 y	94	22.6 (15.3–46.5)	776.3 (697.9–815.8)	211.2 (697.9–815.8)
11–13 y	152	17.3 (4.8–58)	589.6 (530.7–599.7)	211.2 (201.9–242.7)
14–16 y	175	87.5 (21.0–89.4)	505.0 (465.0–551.5)	262.0 (246.3–275.0)
17–19 y	140	87.7 (39.8–113.5)	569.9 (524.7–645.5)	283.8 (258.0–317.0)
20–30 y	672	31.3 (27.2–67.5)	710.1 (659.6–730.2)	291.7 (275.8–302.3)
31–40 y	658	87.5 (49.7–100.9)	560.5 (533.7–582.7)	260.5 (249.1–271.7)
41–50 y	730	74.4 (51.9–79.2)	605.2 (570.2–632.7)	252.5 (239.4–262.9)
51–60 y	797	67.5 (45.1–79.4)	550.1 (511.8–566.9)	241.0 (233.6–249.2)
61–70 y	578	74.8 (44.5–72.7)	520.8 (490.7–538.6)	232.2 (220.0–243.3)
>70 y	368	20.0 (10.7–45.9)	519.1 (489.6–555.1)	252.4 (236.0–267.2)

CI: Confidence interval

($p<0.001$) until median values near 200 mUI/L are observed throughout most of childhood. During the children's period, no gender differences were observed for prolactin level ($p = 0.167$). The sex difference in median prolactin levels became significant ($p<0.001$) from pre-adolescence onwards and will continue until middle-aged adults (51 – 60 y) stage. For young-seniors and senior, no sex-differences were observed ($p<0.001$).

For females and from pre-adolescence, a progressive and significant ($p<0.001$) increase in median serum prolactin levels is observed until young adulthood, followed by a gradual and continuous decrease until young senior age.

For males and from adolescence, a gradual and significant increase ($p<0.001$) in median serum prolactin levels is observed until adulthood, followed by a gradual and continuous decrease until young senior age.

5. Discussion

In this context of falsely elevated prolactin, it was important to re-evaluate the reference intervals used to ensure that they were appropriate for the method used and for our

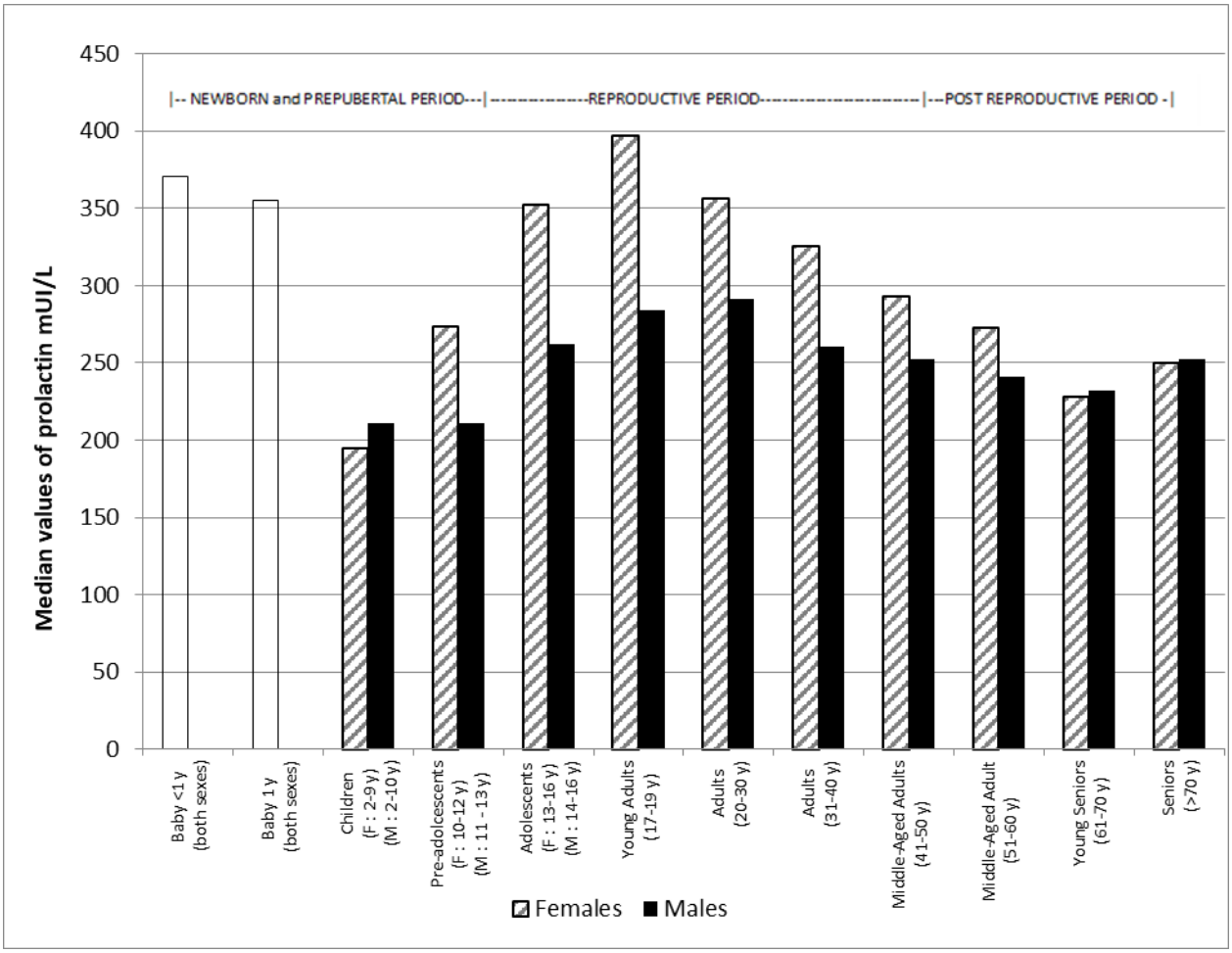

Fig. 1: Median serum prolactin (mUI/L) in men and women of different age groups

population. The Roche Diagnostics data sheet indicated the 95% reference ranges (2.5^{th} – 97.5^{th} percentiles) for serum total prolactin were 86–324 mIU/L and 102–496 mIU/L for males (N=102) and females (N=198), respectively. The manufacturer selected the reference ranges as described in the study by Fahie-Wilson MN et al.[5] In this study, partitioning was limited to only one age group (20 – 63y for females and 20 – 64 y for females), and the median values for women and men were 225 mUI/L and 155 mUI/L respectively. For this same age group, the median values obtained in our study were 10 – 60% higher according to the age and sex of the patients. In 1975, Vekemans M and Robyn C[11] have shown the decline in serum prolactin levels with age in women and the expanding distribution of age groups has proved to be very useful in interpreting the results of prolactin assays. One obvious cause for the decrease in PRL in women could be the lack of estrogens.[8]

Significantly elevated serum prolactin levels, with considerable variation, have been observed during the neonatal period.[12] In our study, after the observation of higher serum prolactin levels in the first year of life, these decrease to median values close to 200 mIU/L during most of childhood.

No gender differences were observed during the children period. The gender distinction became significant from pre-adolescence until the age of 60 suggesting a sexual dimorphic role of prolactin in pituitary, hypothalamus, and hippocampus.[13] For young-seniors and seniors, no sex-differences were observed, and a common prolactin reference interval may be appropriate.

Our results revealed that prolactin levels increased significantly between children and young adults and partitioning with different age groups may be appropriate in interpreting the results of prolactin assays. In female, the prolactin concentration began increasing earlier than in males (Figure 1), which may be explained by an earlier increase in LH and FSH levels.[14] In males, this growth is correlated with the function of prolactin in masculine reproductive physiology. Gill-Sharma[15] has shown the relationship between testosterone and prolactin. Systemic prolactin levels could contribute to the mechanism of chromatin condensation during spermiogenesis.

Our study showed a slight increase in the median values in seniors. According to some authors, it seems that the stress caused by sexual dysfunction in the elderly may induce hyperprolactinemia.[16,17]

6. Conclusion

This study showed a discrepancy between the prolactin reference values proposed by the manufacturer in the data sheet and our results. The median serum prolactin values appeared to be higher, and laboratories should review the reference ranges for gender and different age groups, which may be more appropriate. We hope that this study will allow a better management of hypothalamic-pituitary disorders.

7. Source of Funding

None.

8. Conflict of Interest

Nothing to declare.

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