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Establishment of Reference Ranges for Prolactin in Neonates, Infants, Children and Adolescents

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Summary: Prolactin was determined in the sera of 686 healthy neonates, infants, children and adolescents (age range 5 days to 18 years), using the IMx from Abbott Laboratories. The applied test was a microparticle enzyme immunoassay (MEIA). The proband collective was divided into 9 age groups, and each age group into males and females. In accordance with the recommendations of the International Federation of Clinical Chemistry, the 95% scatter range was taken as the reference range. Only a few reference groups showed a normal *Gauss*ian distribution. In addition to the 50th percentile, the 2.5th and 97.5th percentile were calculated for all reference groups, and the minimal and maximal values were also reported. From the age of 12 years onwards, significant differences were found between males and females. The U-test of *Mann & Whitney* was used to test for significant differences between individual reference groups. Groups showing no significant differences were combined, and the corresponding reference ranges for serum prolactin were then calculated.

Introduction

Serum prolactin is measured in childhood mainly as a means of establishing the presence or absence of increased prolactin secretion.

The causes of hyperprolactinaemia are complex. Particularly important are pituitary tumours or other encroachments on space, which compress the pituitary stem and prevent inhibitory factors from reaching the site of prolactin secretion (1–3).

Excessive prolactin production may not be clearly evident from the basal serum prolactin concentration alone. In such cases, a provocation test is performed, in which prolactin secretion is stimulated by thyrotropin releasing hormone (1).

In view of the numerous modern determination methods for serum prolactin, the reference ranges for any study should be determined with the method adopted for that study (4, 5).

The aim of the investigation was:

1) to determine the reference ranges for serum prolactin in healthy neonates, infants, children and adolescents;
2) to test for significant sex differences in serum prolactin concentration within the reference groups; and
3) to test for significant differences in serum prolactin concentration between the reference groups.

Materials and Methods

Prolactin was determined in the sera of 686 healthy neonates, infants, children and adolescents (age range 5 days to 18 years). In the course of routine screening for hypothyreosis venous blood was taken from 5-day-old neonates. For all other probands blood samples wer taken after written consent was obtained from their parents, who were informed as to the purpose of the tests. The Ethics Commission of the Medical School of Erfurt gave its agreement for this purpose. The age composition

Tab. 1. Age composition of the proband collective for the determination of reference ranges of prolactin in neonates, infants, children and adolescents

Group	Age	n
1 ♂	5th day	70
1 ♀	5th day	70
1	5th day	140
2 ♂	2–12 month	14
2 ♀	2–12 months	11
2	2–12 months	25
3 ♂	2–3 years	18
3 ♀	2–3 years	16
3	2–3 years	34
4 ♂	4–6 years	42
4 ♀	4–6 yeears	23
4	4–6 years	65
5 ♂	7–9 years	44
5 ♀	7–9 years	41
5	7–9 years	85
6 ♂	10–11 years	44
6 ♀	10–11 years	52
6	10–11 years	96
7 ♂	12–13 years	44
7 ♀	12–13 years	45
7	12–13 years	89
8 ♂	14–15 years	39
8 ♀	14–15 years	38
8	14–15 years	77
9 ♂	16–18 years	37
9 ♀	16–18 years	38
9	16–18 years	75

of the proband collective is summarized in table 1. Individuals were included or excluded according to the criteria of *Witt & Trendelenburg* (6), which permit the assembly of a reliable reference sample at a justifiable expense. Only those neonates with a birthweight between 2500 and 4000 g and a full term gestation time between 37 and 40 weeks were admitted to the 5-day-old age group. Neonates with hyperbilirubinaemia were excluded, as well as those born to mothers with acute or chronic illnesses. In sexually mature girls, blood was taken during the first 10 days of the follicular phase of a monthly cycle.

Test material

About 2 ml of blood were taken between 08.00 and 10.00 am, from an arm or skull vein, using safety monovettes from Sarstedt, Nümbrecht. Blood samples were centrifuged immediately for 5 min at 3000 min^{-1}. The serum was removed with a pipette, then frozen at −22 °C until analysed.

Method

Prolactin was determined by a microparticle enzyme immunoassay, using the IMx from Abbott Laboratories. Cross reactivities of the test, as quoted by the manufacturer, are shown in table 2. The stated test sensitivity was 0.6 µg/l. The method was calibrated with the WHO 2nd International Standard 83/562 (1 µg/l prolactin corresponds to 24 mU/l WHO standard).

Tab. 2. Cross reactivities reported by the manufacturer for the test used on the IMx (concentration of each tested substance in brackets)

Test	Cross reactivity
IMx Prolactin	with follitropin (5000 mU/l): none with thyrotropin (20 000 mU/l): none with human chorionic gonadotropin (100 000 U/l): none with lutropin (5000 U/l): none with gonadotropin (1000 µg/l): 0.039% with human placental lactogen (100 000 µg/l): none

Quality control

For the control of precision from day to day, standards (from Abbott Laboratories) of low, intermediate and high concentration were included intermittently in each series. As a measure of the relative methodical error, the arithmetic mean ($\bar{x}$), standard deviation (s) and coefficient of variation (CV) were calculated from the individual results of these control series. Precision in series was monitored once, using calibrators "B" and "E" of low and high concentrations from Abbott Laboratories. Again, the arithmetic mean ($\bar{x}$), standard deviation (s) and the coefficient of variation (CV) were calculated from the individual results.

Statistical evaluation of the results

The results were first presented as separate histograms for each age group and for each sex. The type of distribution was determined with the *Kolmogorov-Smirnov* test. If the resulting error probability was below the stated value of $\alpha = 0.05$, the distribution was assumed to be normal. If the distribution was not normal, the 2.5th, 50th and 97.5th percentiles were determined for that reference group (7). In each age group, the values for prolactin were tested for significant sex differences, using the U-test of *Mann & Whitney*, again using a limiting value of $\alpha = 0.05$ for the error probability. In the absence of a significant sex-related difference, males and females were subsequently treated as a single group. The U-test of *Mann & Whitney* was also used to test for significant differences between age groups, and all groups showing no significant difference were combined. The median value and reference range for serum prolactin were calculated for all the final combinations of reference groups.

The degree of any linear relationship between age and serum prolactin concentration was determined by calculation of the correlation coefficient, r.

Results

Prolactin was determined in the serum of 686 healthy probands (352 males, 334 females). Figure 1 gives an overview of the results for all groups before significance testing. Significant differences between the sexes

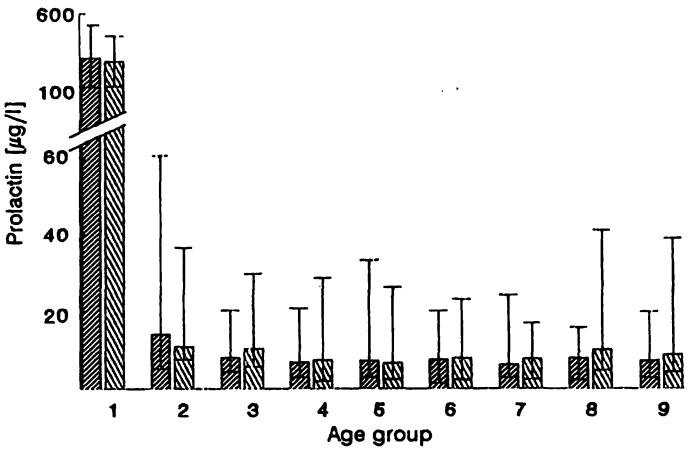

Fig. 1. 50th Percentiles and 95% intervals for the concentrations of prolactin (µg/l) in the serum of age groups 1–9 (see tab. 1).
▨ males; ▧ females.

were found in age groups 7, 8 and 9 ($p = 0.0091$, $p = 0.0012$ and $p = 0.0271$, respectively).

All reference groups were tested for significant differences, using the U-test of *Mann & Whitney*. Although the prolactin values of 12–13-year-old males and the 12–13-year-old females were not significantly different, these two groups were kept separate.

Table 3 shows the new groups formed after significance testing. The median value and reference range for serum prolactin were recalculated for each new group combination.

A correlation analysis was performed for the relationship between proband age and the serum concentration of prolactin. A significant negative correlation was found ($p < 0.001$), i.e. the concentration of serum prolactin decreased with increasing age ($r = -0.5927$).

Quality control

Results of the quality control are shown in table 4. The variation coefficients within series and between series were all less than 8%.

Tab. 4. Results for the control of precision from day to day and in series

	Control serum	n	$\bar{x}$ [µg/l]	s [µg/l]	CV [%]
Control from day to day	Abbott L	18	7.95	0.61	7.67
	Abbott M	41	20.5	1.20	5.86
	Abbott H	21	40.9	2.41	5.89
Control in series	"B" calibrator	23	4.66	0.14	2.94
	"E" calibrator	23	69.2	1.66	2.40

Discussion

For the correct evaluation of the reported serum prolactin concntration, reference ranges must be established. Most modern immunometric assays have so far been used to determine reference ranges for adults, but usually not for children. Childhood reference ranges for serum prolactin, determined with the present method, have not been reported in the literature.

Reported reference ranges (8, 9) for childhood, using other methods, are shown in table 5.

The data shown in table 5 are not comparable with the present results, because:

1) other methods were used;
2) different age classifications were used;
3) the numbers of probands in each age group were not reported;
4) no data were reported on the type of distribution of the reference values;
5) information was sometimes lacking on the reference preparation used for calibration purposes.

In the present study, 686 neonates, infants, children and adolescents (age range: 5 days to 18 full years) were used to determine the reference ranges of serum

Tab. 3. 50th Percentile, 95% interval, minimal value and maximal value for the serum concentration of prolactin in neonates, infants, children and adolescents (values in µg/l)

Age	Sex	n	Median (50th percentile)	Normal (95% scatter range (2.5–97.5th percentiles)	Minimum	Maximum
5 days	♂/♀	140	238	102 –496	95.7	600
2–12 months	♂/♀	25	12.4	5.30– 63.3	5.3	63.3
2– 3 years	♂/♀	34	9.85	4.40– 29.7	4.40	29.7
4–11 years	♂/♀	246	7.20	2.63– 21.0	1.3	33.9
12–13 years	♂	44	6.20	2.84– 24.0	2.8	25.7
12–13 years	♀	45	7.80	2.52– 16.9	2.40	17.0
14–18 years	♂	76	7.35	2.76– 16.1	2.30	19.7
14–19 years	♀	76	9.50	4.20– 39.0	4.20	41.2

Tab. 5. Reference ranges reported in the literature for the concentration of prolactin in serum

Author	Method	Age groups	No. of probands	Type of distribution and scatter range	Normal range
Sitzmann, 1986 (8)	No data (µg/l)	Neonates, 1st week	No data	No data	76 ± 36
		Age 6 weeks			17 ± 6
		Prepuberty		Median value	11 ± 4
		Adolescence		given ± SD	14 ± 5
	No data	Infants, 1–5 months	No data	No data	10.0 ± 1.9 (6.1–15)
		Children, 1–2 years			6.0 ± 1.0 (2.3–12)
		Girls, 4–17 years		Median value	
	hPRL*) Lewis 203	Puberty stage 1		given ± SD	7.1 ± 1.2 (2.9–18)
		Puberty stage 2			9.0 ± 1.3 (4.6–12)
		Puberty stage 3		No clear data	4.1 ± 1.1 (2.7– 7.4)
		Puberty stage 4		on scatter range	6.9 ± 0.8 (4.8– 9.2)
		Puberty stage 5			7.4 ± 0.8 (3.3–19)
		Boys, 5–7 years			6.0 ± 0.5 (1.7–16)
Plenert & Heine 1984 (9)	RIA (10^3/U/l)	♂ 1 day	No data	No data	6899 ± 3622
		6 days			3358 ± 1171
		12–23 days			1675 ± 700
		3–7 months			312 ± 127
		2 years			290 ± 289
		6–7 years			188 ± 113
		8–9 years			265 ± 116
		10–11 years			110 ± 56
		12–13 years			116 ± 103
		14–16 years			188 ± 199
		♀ 1 day			9640 ± 4711
		6 days			2709 ± 1300
		12–23 days			1851 ± 475
		3–7 months			355 ± 221
		2 years			228 ± 203
		6–7 years			218 ± 172
		8–9 years			251 ± 114
		10–11 years			266 ± 165
		12–13 years			216 ± 153
		14–16 years			241 ± 139

*) human prolactin

prolactin in childhood. The probands were first divided into 18 groups according to age and sex (see tab. 1). The chosen age classification was based on the suggestions of *Egger* et al. (10) and the recommendations of the International Federation of Clinical Chemistry (11). The neonatal age of 5 days was chosen simply because the use of this age for other diagnostic studies (e.g. hypothyreosis screening) means that proband material is readily available. In contrast, no probands were available between the ages of 6 and 30 days. Also, data from the 2–12-month-old male and female infants have limited interpretative value, due to the small numbers of probands in these groups.

The analytical method was a microparticle enzyme immunoassay (MEIA). Advantages of this method are its speed, its requirement for only a small sample volume, and the absence of radioactive isotopes.

Extraordinarily high concentrations of prolactin were found in the sera of 5-day-old neonates. No sex differences were detectable up to the age of 11 years. From the age of 14 years onwards, the serum prolactin concentration was significantly higher in females than in males.

Tab. 6. Reference ranges for prolactin in the serum of neonates, infants, children and adolescents (µg/l)

Males	
5th day	102 –496
2–12 months	5.30– 63.3
2–3 years	4.40– 29.7
4–11 years	2.63– 21.0
12–13 years	2.84– 24.0
14–18 years	2.76– 16.1
Females	
5th day	102 –496
2–12 months	5.30– 63.3
2–3 years	4.40– 29.7
4–11 years	2.63– 21.0
12–13 years	2.52– 16.9
14–18 years	4.20– 39.0

A significant negative correlation was found between age and serum prolactin concentration, i.e. serum prolactin decreased with increasing age. This statistical result, however, is due primarily to the extremely high concentrations measured in 5-day-old neonates.

In accordance with the recommendations of the International Federation of Clinical Chemistry, the 95% scatter range was taken as the normal range. Since the values of most reference groups do not show a normal distribution, the reference range was reported as the 2.5th and 97.5th percentiles, together with the median value (50th percentile).

Where possible, groups showing no significant differences after significance testing were combined. These final group combinations and their respective reference ranges are shown in table 6.

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References

1. Derricks-Tan, J. S. E. (1988) *Grundlagen der Prolaktin-Diagnostik*. 1. Auflage, Schattauer, Stuttgart, New York.
2. Benker, G., Jaspers, C., Häusler, G. & Reinwein, D. (1990) Control of prolactin secretion. Klin. Wochenschr. *68*, 1157–1167.
3. Rosenfield, R. L. (1990) The ovary and female sexual maturation. In: *Clinical Pediatric Endocrinology*. Second Edition (Kaplan, S. A.) W. B. Saunders Company, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo.
4. Haro, L. S., Lee, D. W., Singh, R. N. P., Bee, G., Markoff, E. & Lewis, U. J. (1990) Glycosylated human prolactin: Alterations in glycosylation pattern modify affinity for lactogen receptor and values in prolactin radioimmunoassay. J. Clin. Endocrinol. Metab. *7*, 379–383.
5. Kunkel, S., Rudolf, K., Woltanski, K.-P. & Büttner, S. (1990) Heterologe und homologe Radioimmunoassays für humanes Prolaktion. 1. Mitteilung: Aufbau und Methodenkritik. Zent.bl. Gynäkol. *112*, 535–544.
6. Witt, J. & Trendelenburg, C. (1982) Gemeinsame Studie zur Erstellung von Richtwerten klinisch-chemischer Kenngrößen im Kindesalter. J. Clin. Chem. Clin. Biochem. *20*, 235–242.
7. International Federation of Clinical Chemistry Scientific Committee, Clinical Section Expert Panel on Theory of References Values and International Committee for Standardisation in Haematology. Standing Comittee on Reference Values (1987) Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J. Clin. Chem. Clin. Biochem. *25*: 645–656.
8. Sitzmann, F. C. (1986) *Normalwerte*. 2. Auflage, Hans Marseille, München.
9. Plenert, W. & Heine, W. (1984) *Normalwerte*. 6. Auflage, Verlag Volk und Gesundheit, Berlin.
10. Egger, E., Dummler, W., Neymeyer, H.-G., Schwarze, H. & Scholz, R. (1987) Vorschlag zum Arzneibuch der DDR, Diagnostische Laboratoriumsmethoden, AB (D. L.)-DDR 89. Allgemeines. Referenzbereiche. Zent.bl. Pharm. *126*, 737–747.
11. International Federation of Clinical Chemistry Scientific Committee, Clinical Section Expert Panel on Theory of Reference Values (1987) Approved recommendation (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values. J. Clin. Chem. Clin. Biochem. *25*, 639–644.

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