GH Values In Serum And Blood Spots On Filter Paper Samples In Neonates Until 30 Days Of Life By Electrochemiluminescence (ECLIA).

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Introduction

The Growth Hormone Deficiency (GHD) in newborns is an infrequent condition, which can cause threat to life due mainly to hypoglycemia that begins in the first week of life. Severe neonatal GHD needs a fast diagnosis and the substitution with recombinant human GH for the risk of morbi-mortality.
Castro L et al, JRAEM 2017.

A GH level (whether random or associated with spontaneous hypoglycemia) that distinguishes infants with GHD from those with GH sufficiency in the neonatal periods is not conclusive.
Binder G et al, JCEM 2010.

There are no data of normal values in serum and dried blood spot for the determination of GH commercially available at present. It is important to bear in mind that the studies from which normative data are extracted involve a small number of children, which constitutes an additional limitation in clinical interpretation.

Aims

The aims of this study were to compare and correlate the GH values in serum and whole blood spots on filter paper samples in neonates until 30 days of life.
To establish GH reference values in healthy newborn until 15 days of life.
To analyzed the correlation between GH concentrations in serum and whole blood spot and with hormones of the hypothalamic-pituitary-gonadal and adrenal axis.

Subjects and Methods

- Whole blood and serum samples were collected from neonates and infants who were attended to perform the screening neonatal.
- We analyzed 281 serum and whole blood spots samples obtained from AGA neonates between 2-30 days of life
  (F: 144; M:137)
  - 2-15 days n=216
  - 16-30 days n=65
  - In addition, we analyzed 20 infants between 30-60 days of life.
  - The elution technique was performed based on the protocol described by Langkamp M.

Growth Hormone & IGF Research 2008, 18: 526-532.
McDade T. Am J Hum Biol 2014.

- Serum and eluted from filter paper GH concentrations were measured by ECLIA Roche C600 (calibrated against the 2nd International Standard Code 98/574).
  (LD: 0.03 ng/mL, LQ: 0.05 ng/mL)
  - VC% intra-assays= 1.5 (0.69 ng/mL); 1.3 (7.91 ng/mL)
  - VC% inter-assays= 2.8 (0.69 ng/mL); 2.7 (7.91 ng/mL)
- A correlation between both techniques in parallel (serum and filter paper samples) were established.
- Means, SD and 5.0- 95.0 percentiles were calculated.
- The comparison between the serum and eluted from filter paper GH measurements and hormones of the hypothalamic-pituitary-gonadal and adrenal axis were performed by Spearman correlation coefficient.

Validation of hGH measurement in dried blood spots:
Freshly spotted filter paper samples were dried at room temperature and subsequently stored at 4° C until processing. The filter disks were then punched out and underwent extraction which different extraction buffer volume and incubation times. No significant difference between the different eluates were finding.

Recombinant hGH was immediately added after an EDTA blood sample was collected. We supplemented 1 ml of blood with various amounts of hGH; and whole blood was pipetted on to the filter paper, dried for at least 2 h at room temperature and then used for hGH measurement. Recovery in concentrations of 10 and 20 ng/mL were 89.5% and 90%, respectively.

Results

The GH concentrations of the corresponding whole blood spots and serum samples are shown in figure 1.

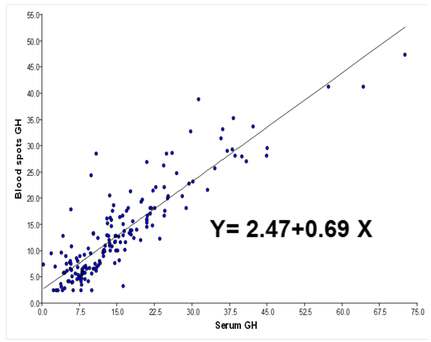

Fig 1: Correlation between serum GH and blood spot GH samples (n=160)

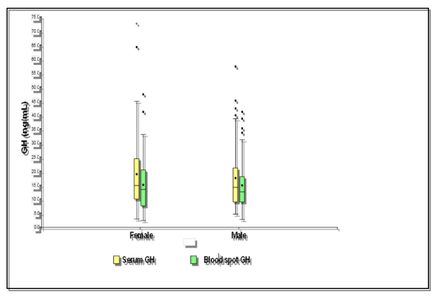

Fig 2: Box plot of GH values (ng/mL) in neonates by gender

The correlation obtained between both techniques in parallel measurements was r2=0.85

We did not find significant difference according to gender

Table 1. Serum and blood spot GH values (ng/mL) in neonates between 2-15 days of life.

	CA (Days)	n	Means	SD	P (5)	P (95)
Serum	2-5	105	20.81	15.80	6.30	42.30
	6-15	60	8.78	4.34	3.29	15.19
Blood spot	2-5	108	17.58	9.95	5.68	33.60
	6-15	69	10.31	5.88	3.00	21.02

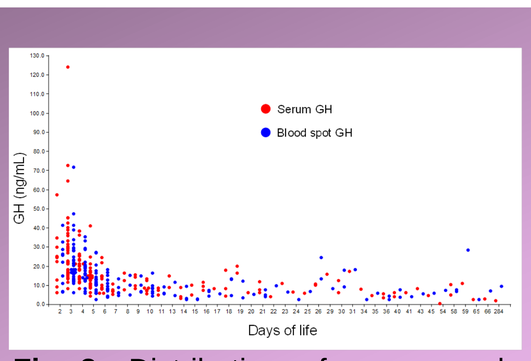

Fig 3: Distribution of serum and blood spot GH values (ng/mL) in neonates until 60 days of life.

Table 2. Serum GH (ng/mL) in neonates by gender.

Days	Gender	n	Means	SD
2-5	F	89	16.92	12.25
16-30	F	35	10.55	4.58
31-60	F	10	4.67	2.75
2-5	M	92	17.27	15.70
16-30	M	30	7.72	3.75
31-60	M	10	6.82	4.57

Table 3. Blood spot GH (ng/mL) in neonates by gender.

Days	Gender	n	Means	SD
2-5	F	89	16.28	10.19
16-30	F	35	10.41	6.01
31-60	F	10	7.02	5.25
2-5	M	92	16.40	11.03
16-30	M	30	10.99	7.78
31-60	M	10	9.12	8.08

Table 4. Correlation obtained between serum GH and hormones of the hypothalamic pituitary-gonadal and adrenal axis

		Female		Male	
Variable	N	Spearman Correlation	p	Spearman Correlation	p
CA	90	-0.67	<0.0001	-0.60	<0.0001
BW	90	-0.04	0.7195	-0.03	0.7916
FSH	74	-0.51	0.0001	-0.37	0.0012
LH	74	-0.22	0.0567	-0.09	0.4697
To	74	0.23	0.0520	0.24	0.0374
E2	74	0.04	0.7105	0.13	0.2794
Co	74	0.34	0.0027	0.35	0.0023

Table 5. Correlation obtained between blood spots GH and hormones of the hypothalamic-pituitary-gonadal and adrenal axis

		Female		Male	
Variable	n	Spearman Correlation	p	Spearman Correlation	p
CA	84	-0.78	<0.0001	-0.68	<0.0001
BW	84	-0.07	0.5232	-0.09	0.4340
FSH	76	-0.40	0.0003	-0.31	0.0067
LH	76	-0.38	0.0006	-0.35	0.0019
To	76	0.20	0.0880	0.08	0.4743
E2	76	0.08	0.4742	0.19	0.1066
Co	76	0.19	0.1001	0.21	0.0653

Discussion and Conclusions

- ✓In agreement with different reports, our results showed high average GH levels in the first few days of life. Human GH secretion is pulsatile from the very beginning, however, newborn screening card spotted with blood during the first week of life, when neonatal hypersomatotrophism is present, provides such high levels that, even at the nadir of GH pulsatility a basal value could contribute to detect GHD accurately.
- ✓The good correlation obtained between both type of samples would indicate that the measurement of GH in dried blood spot samples is an appropriate and reliable method which can be incorporated into the neonatal GHD diagnosis.
- ✓The newborn screening samples may be a valuable resource for retrospectively assessing GH sufficiency if this neonatal window has passed.