Measurement of Leptin by RIA Versus MIA in a Population of Healthy Newborns

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Background: Assays based on multiplex immunoassay (MIA) technology have demonstrated advantages over enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA). Its acceptance depends on how well it performs in comparison to older techniques. The aim is to compare the results of leptin using RIA versus MIA. **Methods:** We analyzed 81 samples of umbilical cord blood of healthy term newborns by RIA and MIA. **Results:** The con-

cordance correlation coefficient was 0.158 (95% CI 0.10–0.21). Pearson's correlation coefficient was 0.6651 (95% CI 0.52–0.77; $P < 0.0001$). In the Bland–Altman plot, concordance is acceptable because most of the measurements are within a mean of ± 1.96 SD. **Conclusions:** As shown by the Bland–Altman plot, there is concordance by both methods, but with a weak correlation. J. Clin. Lab. Anal. 30:254–257, 2016. © 2015 Wiley Periodicals, Inc.

Key words: leptin; newborns; multiplex immunoassay; radioimmunoassay; correlation

INTRODUCTION

Obesity research has produced new techniques for the study of expression of a large number of bioactive peptides secreted by adipose tissue, known as adipokines. Among these are leptin, a hormone involved in the control of energy homeostasis (1,2).

The studies by Barker et al. (3) in the 1980s established that the incidence of some diseases in adulthood related to obesity, such as stroke, diabetes mellitus, and dyslipidemia, is associated with the intrauterine environment (the Barker hypothesis or programming theory). The induction of different phenotypes by environmental variations in early life is associated with varying degrees of metabolic disease (3); hence, the importance of knowing the expression of obesity-related adipokines through umbilical cord blood analysis.

Measurement of these adipokines in epidemiological studies using techniques based on enzyme-linked immunosorbent assays (ELISAs) and radioimmunoassays (RIAs) has been widely accepted, but it incurs a significant amount of time, cost, and sample volume, limiting the ability to examine samples in vulnerable populations such as children, as well as systematically evaluating the effects of clinical interventions in this age group (4,5). Assays based on multiplex immunoassay (MIA) technology

have the advantage that with small sample volumes it is possible to detect multiple analytes, and to produce an ad hoc cluster for the study of inflammatory diseases, sepsis, diabetes, metabolic syndrome, and other pathologies (2,6) in populations with specific characteristics, thereby reducing costs (7).

When comparing the introduction and adoption of new techniques with that of old ones, a gap becomes evident. Currently, there are reports comparing RIA and ELISA versus MIA in pathological situations (8,9); thus additional comparisons, especially in reference populations, allow integration of this technology to existing techniques with the previously cited advantages (10). The aim of our study is to compare the results of leptin levels in

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TABLE 1. Method Comparison Between RIA and MIA

	<i>n</i> = 81	Sample analyzed			Regression analysis			Assay parameters		
		Mean	SD	Range	Slope	Intercept	<i>R</i>	Standard range	Measurement range	Detection limit (sensitivity, ng/ml)
Leptin (ng/ml)	RIA	8.35	7.76	0.96–44.83	–1.1177	–3.84	0.6651	0.5–100	0.5–100	0.437
	MIA	38.24	22.52	3.26–104.28				0.016–250	0.016–250	0.0854

Regression analysis performed by standardized principal component analysis. The concentration range of the standards with the manufacturer's recommended serial dilution. Limit of detection (determined from blank measurements).

R, correlation coefficient.

umbilical cord blood of a sample of healthy term newborns using a commercial kit for RIA and another with MIA technology.

MATERIALS AND METHODS

We conducted a prospective study from January 2006 to December 2007 at the Universidad Autónoma de Nuevo León, Hospital Universitario "José Eleuterio González" in Monterrey, Mexico, in a cohort of healthy term infants with appropriate weight for gestational age according to the Z-score classification of the World Health Organization (WHO) (11). We analyzed 81 samples of umbilical cord blood obtained immediately after birth and centrifuged at 3,000 rpm. The serum was separated into aliquots for freezing and storage at –20°C until analysis by RIA and MIA with the intention of correlating the results of both techniques. Samples were thawed only at the time of analysis. The study was approved by the Research Ethics Committee of the Hospital Universitario "José Eleuterio González" of the Universidad Autónoma de Nuevo León. Parents provided written informed consent prior to the collection of data and blood samples.

METHODOLOGY FOR THE DETERMINATION OF LEPTIN

Leptin was measured by RIA using the Human Leptin Radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO) on the Packard Gamma Counter (Global Medical Instrumentation, Ramsey, MI), and the assay sensitivity is 0.437 ng/ml according to the manufacturer's instructions. Leptin was measured by MIA using a Human Serum Adipokine (Panel B) kit (Millipore, St. Charles, MO) on a Luminex 200 (Luminex Corporation, Austin, TX) and the sensitivity of the assay is 85.4 pg/ml according to the manufacturer's instructions. To compare the two methods, the results of MIA units (pg/ml) were converted to the units reported by the RIA method (ng/ml).

RESULTS

In 81 of 99 cases, a serum sample from umbilical cord was available for processing by both methods of measurement. Evaluation of leptin by both methods is shown in Table 1.

The intraassay variation coefficients in our study were 5.17% and 9.78% for RIA and MIA, respectively; interassay coefficients were 6.4% and 15%, respectively, according to the manufacturer's specifications (12, 13).

A median of 5.85 ng/ml with an interquartile range of 3.26–7.94 was found in measurements of leptin by RIA. With MIA, the median was 34.68 ng/ml with an interquartile range of 21.33–50.74. All analyzed samples had values above assay detection limits.

The concordance correlation coefficient was 0.158 (95% CI 0.10–0.21). Pearson's correlation coefficient was 0.6651 (95% CI 0.52–0.77; $P < 0.0001$). To characterize the relationship between the two tests, a Bland–Altman plot was made to contrast the differences between mean leptin by RIA and MIA versus the difference between the two measurements (14). The concordance is acceptable because most of the measurements are within mean ± 1.96 SD (Fig. 1).

DISCUSSION

Access to new multiplex assay technology, where several analytes can be determined simultaneously, including adipokines, has shown advantages over traditional ELISA and RIA techniques, since using small sample volumes provides higher yields, thus making this a possible common test platform in the future and a breakthrough in clinical research (5, 7). It is difficult to compare our results with previously published reports because these were not made with umbilical cord blood of healthy newborns but with blood from adults with various comorbidities and using different methods (ELISA vs. MIA) for comparison.

In our study, the concentration range of the analyzed samples was wider for MIA than for RIA, unlike that reported by Loo et al. (7) where their concentration range

Fig. 1. (A) Bland-Altman plot of the correlation between subtraction and mean leptin (ng/ml) by RIA and MIA. (B) Bland-Altman plot representing subtraction of the mean and its correlation with leptin by RIA and MIA.

was higher for ELISA than for MIA. In our study, we found substantial differences in the estimation of the leptin concentration between both methods; the mean absolute estimation of leptin is 4.57 times greater in MIA than in RIA, while Loo et al. (7) reported a mean leptin 1.8 times higher by ELISA than by MIA. These variations are probably due to the use of different pairs of antibodies. In addition, the presence of heterophilic antibodies is another possible explanation for the observed discrepancies between the two methods, since it is known that these antibodies may be present in 40% of the normal population (15). It is beyond the scope of this study to point out the gold standard of the measurements and which method provides results that are closer to current blood levels.

Similar to Martos-Moreno et al. (4) and Liu et al. (5), in our study, leptin detection limits were lower for MIA in comparison with RIA and ELISA, respectively. In contrast with Loo et al. (7), we were able to detect leptin in all analyzed samples. This is particularly important in our study population because they are healthy newborns where low leptin concentrations in umbilical cord blood are expected without associated physical stress conditions, as demonstrated in our previous study (16).

Possible causes of low correlation coefficients between the two methods have been previously proposed by Martos-Moreno et al. (4), Schipper et al. (17), Liu et al. (5), who cite the dissimilarity in pairs of antibodies used and the extremely low plasma concentrations of analyzed cytokines.

Using the same method for the determination of an analyte in populations with long-term monitoring is best as stated by Elshal et al. (18). However, with the advent of new technologies with additional advantages, the trend is to replace conventional technologies; a possible practical solution to this problem could be to generate conversion equations for the determination of analytes by the various methods while both technologies coexist.

To our knowledge, MIA is not sufficiently mature to be used in everyday clinical practice. However, its safety, by requiring small sample volumes, is ideal for pediatric patients, and its cost-effectiveness with reduction in personnel, materials, lower cost of consumables, and scalability of technology are ideal for current use in research (19).

In conclusion, as shown by the Bland-Altman plot, there is concordance with both methods, but it is not possible to compare the obtained results because of the weak correlation found.

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ABBREVIATIONS

ELISA = enzyme-linked immunosorbent assay
MIA = multiplex immunoassay
RIA = radioimmunoassay
WHO = World Health Organization.

REFERENCES

1. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004;89:2548–2556.
2. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: An endocrine organ. *Arch Med Sci* 2013;9:191–200.
3. Barker DJ, Hales CN, Fall CH, et al. Type 2 (non-insulin dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): Relation to reduced fetal growth. *Diabetologia* 1993;36: 62–67.
4. Martos-Moreno GA, Burgos-Ramos E, Canelles S, Argente J, Barrios V. Evaluation of a multiplex assay for adipokine concentrations in obese children. *Clin Chem Lab Med* 2010;48:1439–1446.

5. Liu MY, Xydakis AM, Hoogveen RC, et al. Multiplexed analysis of biomarkers related to obesity and the metabolic syndrome in human plasma, using the Luminex-100 system. *Clin Chem* 2005;51(7):1102–1109.
6. Briana DD, Malamitsi-Puchner A. Intrauterine growth restriction and adult disease: The role of adipocytokines. *Eur J Endocrinol* 2009;160:337–347.
7. Loo BM, Marniemi J, Jula A. Evaluation of multiplex immunoassays, used for determination of adiponectin, resistin, leptin and ghrelin from human blood samples, in comparison to ELISA assays. *Scand J Clin Lab Invest* 2011;71:221–226.
8. Murdolo G, Herder C, Wang Z, Rose B, Schmelz M, Jansson PA. In situ profiling of adipokines in subcutaneous microdialysates from lean and obese individuals. *Am J Physiol Endocrinol Metab* 2008;295:E1095–E1105.
9. Djoba-Siawaya JF, Roberts T, Babb C, et al. An evaluation of commercial fluorescent bead-based luminex cytokine assays. *PLoS One* 2008;3:e2535.
10. Vignali DAA. Multiplexed particle-based flow cytometric assays. *J Immunol Methods* 2000;243:243–255.
11. de Onis M, Blossner M. The World Health Organization Global Database on Child Growth and Malnutrition: Methodology and applications. *Int J Epidemiol* 2003;32:518e526.
12. Human leptin RIA kit (125 tubes cat.# HL-81 HK). Available at: http://www.emdmillipore.com/US/en/product/Human-Leptin-RIA,MM_NF-HL-81HK#anchor_REF.
13. Human serum adipokine (panel B) kit (96-well plate assay cat# HADK2-61K-B). Available at: <http://www.emdmillipore.com/US/en>.
14. Myles PS. Using the Bland-Altman method to measure agreement with repeated measures. *Br J Anaesth* 2007;99:309–311.
15. Dossus L, Becker S, Achaintre D, Kaaks R, Rinaldi S. Validity of multiplex-based assays for cytokine measurements in serum and plasma from “non-diseased” subjects: Comparison with ELISA. *J Immunol Methods* 2009;350:125–132.
16. Treviño-Garza C, Bosques-Padilla FJ, Estrada-Zúñiga CM, et al. Typical leptin fall is mitigated by breast-feeding in infant females. *Arch Med Res* 2010;41:373–377.
17. Schipper HS, de Jager W, van Dijk MEA, et al. A multiplex immunoassay for human adipokine profiling. *Clin Chem* 2010;56:1320–1328.
18. Elshal MF, McCoy P. Multiplex bead array assays: Performance evaluation and comparison of sensitivity to ELISA. *Methods* 2006;38:317–323.
19. Ray CA, Bowsher RR, Smith WC, et al. Development, validation, and implementation of a multiplex immunoassay for the simultaneous determination of five cytokines in human serum. *J Pharm Biomed Anal* 2005;36:1037–1044.