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Biotin interference with first trimester biochemical markers for fetal aneuploidies

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Objective

Biotin is a water soluble vitamin, that functions as a coenzyme for carboxyl transfer by five carboxylases involved in many metabolic processes. An indeterminate number of pregnant women take vitamin supplements, whose concentration in biotin is unknown to us. It has been demonstrated that for biotin dosing regimens up to of 5 mg/day and 10 mg/day, serum biotin levels of 41 ng/mL and 91 ng/mL are reached after 3 days, respectively; the biotin serum concentration falls to 30 ng/mL 3.5 and 8 hours after the intake, respectively. Exogenous Biotin has the potential to interfere with streptavidin-biotin based on capture techniques like we use in our laboratory for the determination of the free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), for the calculation of the risk index of congenital anomalies of the first trimester. The aim of this study is to evaluate which concentration of biotin in serum interferes with first trimester biochemical markers.

Methods

The study was designed in accordance with the clinical and laboratory standards institute (CLSI) document EP7-A22. For each analyte, fresh serum samples were obtained from 30 healthy pregnant women in different gestational age. Serum samples were frozen at -20oC and stored for 4 weeks, until its use. From the thawed samples, pools of sera were prepared to obtain three levels of concentration. All samples were analyzed in the same analytical series. Biotin stock solution: starting from a biotin solution of 4.6 mg/mL (Medbiotin Fuerte® Reig Jofre lab.), a stock solution of 20 µg/mL was prepared by dilution in diluent universal (roche diagnostics). Preparation of the control and test samples: for each analyte and pool, samples with different concentrations of biotin were prepared by adding Stock solution, to obtain final concentrations of 10, 20, 30, 40, 50, 100, 200, 500 and 1000 µ/L of Biotin. An aliquot of the initial pool was used as a basal control sample. Instrument, reagents and controls: free β-hCG and PAPP-A were measured on a cobas e601 analyzer (roche diagnostics) using a sandwich electrochemiluminescence immunoassay (ECLIA). Free β-hCG CalSet and PAPP-A CalSet were used as calibrators. Precicontrol Maternal Care (roche diagnostics) at three concentration levels was used as internal control. Interference criteria: The deviation from the baseline value above 2.5 SD of our method was defined as the interference criterion. Number of replicates: Adequate replication is required so that the test is performed with sufficient power to detect clinically significant interference, and with a sufficient confidence level to recognize when no clinically important bias exists. As indicated on NCCLS EP7-A2, for a dmax/SD = 2.5 --> number of replicates = 5.

Results

The interference effects of different concentrations of biotin on the detection of free β-hCG and PAPP-A are presented below in the tables.

Conclusion

In the present study we wanted to verify the instructions of the manufacturer, which indicates the absence of interference in free β-hCG and PAPP-A measure at plasma concentrations of biotin below 30 µg/L. This has been confirmed for the PAPP-A, which at biotin levels of 30 µg/L or higher shows significant negative interference. Although methodological problems have not allowed us to evaluate the results of the intermediate level of free β-hCG, the results indicate that negative interference occur with higher biotin concentrations (between 100 and 200 µg/L, depending on the analyte level). The impact of these interferences in the final risk of aneuploidy calculated is difficult to evaluate, since it will depend not only on the magnitude of the interference, but on the level of the biochemical marker (free β-hCG and PAPP-A), the week of gestation and other factors included in the calculation. These results would support the need to have a good communication between the laboratory and clinicians when using streptavidin-biotin assays, taking into account if a pregnant woman is taking biotin supplements, and the dosing regimen. In this case, the extraction of the sample should be done after a washout period sufficient to allow the biotin level to fall below 30 µg/L.

Table 1. Results obtained for Free β-hCG

Base pool Free β-hCG ng/mL	Biotin Concentration µg/L	Mean Concentration Free β-hCG measured ng/mL	Method DS ng/mL	d_{max} ng/mL	Deviation from the baseline value ng/mL	Interference judgment
17.4	10	17.5	0.72	1.8	0.1	No
	20	17.5			0.1	No
	30	17.4			0.0	No
	40	17.4			0.0	No
	50	17.2			-0.2	No
	100	16.7			-0.7	No
	200	15.0			-2.4	Yes
	500	8.6			-8.8	Yes
	1000	1.7			-15.6	Yes
37.1	10	Methodological issue	1.72	4.3	Methodological issue	
	20					
	30					
	40					
	50					
	100					
	200	1.4			-48.2	Yes
	500	0.4			-49.3	Yes
	1000	0.2			-49.5	Yes
103.2	10	101.6	4.36	10.91	-1.7	No
	20	102.1			-1.2	No
	30	100.6			-2.6	No
	40	99.2			-4.1	No
	50	99.7			-3.5	No
	100	92.5			-10.92	Yes
	200	81.4			-21.8	Yes
	500	34.7			-68.5	Yes
	1000	8.5			-94.7	Yes

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	50	17.2			-0.2	No
	100	16.7			-0.7	No
	200	15.0			-2.4	Yes
	500	8.6			-8.8	Yes
	1000	1.7			-15.6	Yes
37.1	10	Methodological issue	1.72	4.3	Methodological issue	
	20					
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	50					
	100					
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103.2	10	101.6	4.36	10.91	-1.7	No
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	40	99.2			-4.1	No
	50	99.7			-3.5	No
	100	92.5			-10.92	Yes
	200	81.4			-21.8	Yes
	500	34.7			-68.5	Yes
	1000	8.5			-94.7	Yes

Table 2. Results obtained for PAPP-A

Base pool PAPP-A mUI/L	Biotin Concentration µg/L	Mean Concentration PAPP-A measured mUI//L	Method DS mUI/L	d_{max} mUI/L	Deviation from the baseline value mUI/L	Interference judgment
153.2	10	149.3	7.97	19.9	-3.8	No
	20	143.5			-9.6	No
	30	135.4			-17.8	No
	40	131.3			-21.8	Yes
	50	131.5			-21.6	Yes
	100	107.9			-45.3	Yes
	200	73.0			-80.2	Yes
	500	20.0			-133.1	Yes
	1000	7.7			-145.5	Yes
1050.4	10	1009.7	36.4	91	-40.7	No
	20	979.9			-70.5	No
	30	930.2			-120.2	Yes
	40	917.3			-133.1	Yes
	50	894.3			-156.1	Yes
	100	731.3			-319.1	Yes
	200	504.1			-546.3	Yes
	500	140.1			-910.3	Yes
	1000	52.6			-997.8	Yes
2785.4	10	2649.4	102	255	-136.0	No
	20	2688.6			-96.8	No
	30	2530.4			-255	Yes
	40	2449.6			-355.8	Yes
	50	2388.6			-368.8	Yes
	100	1932.8			-852.6	Yes
	200	1287.0			-1498.4	Yes
	500	467.4			-2318.0	Yes
	1000	142.5			-2642.9	Yes