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TECHNICAL NOTE

PATHOLOGY AND BIOLOGY

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Postmortem Measurement of Human Chorionic Gonadotropin in Vitreous Humor and Bile

ABSTRACT: Postmortem human chorionic gonadotrophin (HCG) blood assay can confirm postmortem diagnosis of pregnancy or document situations in which HCG levels are elevated. In some cases, however, blood sampling is not possible at autopsy. In this study, HCG was quantified by enzyme-linked fluorescent assay (ELFA) in the bile (n = 5), vitreous humor (n = 4), and postmortem blood (n = 4) of five pregnant women. There were no false negatives in the pregnant subjects (n = 5) or false positives in controls (n = 34), enabling this test to be recommended for routine use in forensic contexts in which the detection of elevated HCG levels could be of interest.

KEYWORDS: forensic science, forensic diagnosis, human chorionic gonadotropin, bile, vitreous humor

Human chorionic gonadotropin (HCG) is a 46.7-kDa glycoprotein hormone with an alpha subunit of 92 amino acids and a beta subunit of 145 (1,2). Table 1 presents the various uses of HCG assay (3–10). HCG also stimulates testosterone secretion and has been banned in male athletes by the International Olympic Committee (IOC) (11). The very slight physiological release of HCG is not enough to render the usual tests positive (12). In forensics, it was demonstrated 20 years ago that beta subunit of HCG can be assayed from blood stains in pregnant women (13). Certain authors have also assayed postmortem HCG in blood and urine to document observations of choriocarcinoma (14,15); in this pathology, blood HCG levels would seem to be comparable antemortem and postmortem (16). To the best of our knowledge, there have been no previous reports of HCG assay in other forensic matrices. The present study therefore sought to assess the feasibility of postmortem HCG assay from blood and two alternative matrices—vitreous humor and bile—using the routine enzyme-linked fluorescent assay (ELFA).

Materials and Methods

Study Population

Thirty-nine forensic autopsy cases were included in the study. The hypothetically HCG-positive cases (n = 5), reported in Table 2, were women who had died while pregnant or early after delivery. The hypothetically HCG-negative cases (n = 34) reported in Table 3 were subjects of childbearing age not known to be pregnant (n = 10) or in whom pregnancy was physiologically impossible (children under the age of 6 [n = 6], postmenopausal women [n = 14], and men [n = 4]). None of the HCG-negative cases were known to have a condition that would result in an endogenous elevation of HCG or to have undergone treatment with HCG, as described in Table 1. For each case, at least one of the study matrices—blood (cardiac or femoral), bile or vitreous humor—was available.

Analysis

HCG was quantified by ELFA on a VIDAS® instrument (BioMérieux, Marcy-l'Etoile, France). The assay principle was combining an enzyme immunoassay sandwich method with a final fluorescent detection. The conjugate was an alkaline phosphatase-labeled monoclonal anti-HCG immunoglobulin (mouse). The conjugate enzyme was catalyzing the hydrolysis of the substrates (4-methyl-ombelliferyl phosphate) into a fluorescent product

TABLE 1—*Main uses of human chorionic gonadotropin (HCG) assay (3–10).*

Intrauterine pregnancy diagnosis and monitoring
Extrauterine pregnancy diagnosis
Down's syndrome screening
Management of hydatiform mole and persistent trophoblastic disease
Female IVF
Male sterility treatment
Management of patients with nonseminomatous germ cell tumors and trophoblastic disease

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TABLE 2—*Postmortem femoral blood, vitreous humor, and bile HCG concentrations in pregnant women.*

					HCG (mIU/mL)			
							Vitreous	
Subjects	Age	Cause of Death	Gestational Age	Autopsy Interval	Blood (Femoral)	Bile	Left	Right
No 1	41	Sudden cardiac death	32 weeks	24 h	6520	2374	172	144
No 2	38	Sudden cardiac death	8 weeks	<24 h	Not collected	3392	Not collected	Not collected
No 3	30	Pulmonary embolism; death 4 hrs after delivery	Term	24 h	2272	13 509	426	407
No 4	28	Pulmonary embolism	32 weeks	Unknown (putrefied)	110	6	28	51
No 5	25	Delivery hemorrhage	37 weeks	24 h	6517	1127	47	69

HCG, human chorionic gonadotropin.

(a-methyl-umbelliferone), the fluorescence of which was measured at 450 nm. The procedures were performed according to the manufacturer's instructions for serum and plasma from living persons: after centrifugation, 100 μL of sample (blood, bile, or vitreous humor) was analyzed. For vitreous humor, analysis was performed for each eye separately in hypothetically positive samples and by pooling both eyes in control subjects. If the HCG concentration was above 1600 mIU/mL, dilution was performed using a diluent provided by the manufacturer. Detection limit was ≤2 mIU/mL. Test was considered positive if the HCG concentration was higher than 4 mIU/mL.

Results and Discussion

In all matrices, all samples from the pregnant women were HCG-positive (Table 2), while no control tests were positive, whichever the matrix (Table 3).

In human postmortem diagnosis, only a few hormones have so far been investigated in vitreous humor; these are: TSH, FSH, LH, and prolactin by Chong et al. (17); free thyroxine (FT3), free triiodothyronine (FT4), and thyroid stimulating hormone (TSH) by Edston et al. (18). In bile only insulin has been studied (19,20). The present study demonstrates that elevated human HCG can be

TABLE 3—*Vitreous humor and bile HCG concentrations in hypothetically HCG-negative population.*

					HCG (mUI/mL)	
Subjects	Age	Sex	Cause of Death	Autopsy Interval	Bile	Vitreous Humor
No 1	0.9	F	SIDS	<24 h	<2.00	<2.00
No 2	1.1	F	SIDS	<24 h	<2.00	<2.00
No 3	1.5	F	Mechanical asphyxia	<24 h	<2.00	<2.00
No 4	2	F	Burns	<24 h	<2.00	<2.00
No 5	3	F	Burns	<24 h	<2.00	Not collected
No 6	4	F	Burns	<24 h	<2.00	<2.00
No 7	22	F	Sudden cardiac death	<24 h	<2.00	<2.00
No 8	26	F	Hanging	<24 h	<2.00	<2.00
No 9	28	F	Gunshot death	<24 h	<2.00	<2.00
No 10	31	F	Sudden cardiac death	<24 h	<2.00	<2.00
No 11	34	F	Poisoning	<24 h	<2.00	<2.00
No 12	35	F	Fall from height	<24 h	<2.00	<2.00
No 13	37	F	Hanging	<24 h	<2.00	<2.00
No 14	37	F	Sudden cardiac death	<24 h	<2.00	<2.00
No 15	40	F	Disseminated intravascular coagulation	<24 h	<2.00	<2.00
No 16	41	F	Poisoning	Unknown (putrefied)	<2.00	Not collected
No 17	55	F	Fall from height	<24 h	<2.00	<2.00
No 18	55	F	Sudden cardiac death	48 h	<2.00	<2.00
No 19	61	F	Sudden cardiac death	<24 h	<2.00	<2.00
No 20	61	F	Knife wounds	48 h	<2.00	<2.00
No 21	62	F	Blunt cranio-cerebral injuries	5j	<2.00	<2.00
No 22	62	F	Acute subarachnoid hemorrhage	<24 h	Not collected	<2.00
No 23	63	F	Drowning	Unknown (putrefied)	<2.00	<2.00
No 24	64	F	Fall from height	<24 h	<2.00	<2.00
No 25	64	F	Poisoning (dextropropoxyphene)	<24 h	<2.00	<2.00
No 26	64	F	Poisoning (tianeptine)	Unknown (putrefied)	<2.00	<2.00
No 27	64	F	Mechanical asphyxia	<24 h	Not collected	<2.00
No 28	68	F	Subdural hematoma in antivitamin K drugs overdose	<24 h	<2.00	Not collected
No 29	76	F	Mechanical asphyxia	72 h	<2.00	<2.00
No 30	78	F	Drowning	Unknown (putrefied)	<2.00	Not collected
No 31	5	M	Burns	<24 h	<2.00	Not collected
No 32	17	M	Knife wounds	<24 h	<2.00	Not collected
No 33	17	M	Overdose (MDMA)	<24 h	<2.00	<2.00
No 34	18	M	Electrocution	<24 h	<2.00	<2.00

HCG, human chorionic gonadotropin.

detected in autopsy samples of blood, bile, and vitreous humor, with a standard kit designed for use with serum or plasma from living patients. The lowest HCG concentrations were found in the samples from subject 4; this was the only case where the blood HCG level was less than expected from the number of weeks of amenorrhea. Although the possibility that an in-utero death had occurred before the mother died, this case did raise the question of the impact of putrefaction on test results, by HCG degradation or the production of interfering substances affecting test results. As previously reported in the case of other hormones (18), concentrations were lower in vitreous humor than in blood, and the two did not correlate. There were no false positives among the negative controls, indicating good test specificity.

In conclusion, the absence of false positives would seem to indicate that the specificity of this postmortem assay is sufficient to determine doubtful pregnancies and to identify other situations in which HCG levels are elevated. A larger sample of positive cases is building up. It would make it possible to determine the sensitivity of this assay when compared to antemortem blood testing, and to study the stability of HCG in postmortem samples.

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