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A SHORT OVERVIEW OF HUMAN CHORIONIC GONADOTROPIN AS A FORENSIC BIOMARKER

Mariana Vieru[1,2], Anca Angela Simionescu[1,3,*], Speranța Schmitzer[1], Florin-Dan Popescu[1,2]

[1]"Carol Davila" University of Medicine and Pharmacy, [2]"Nicolae Malaxa" Clinical Hospital, [3]"Filantropia" Clinical Hospital, Department of Obstetrics and Gynecology, Bucharest, Romania

Abstract: Human chorionic gonadotropin (hCG) may be determined in legal medicine by several immunoassays, including various specimens from women with intrauterine and ectopic pregnancies and patients with choriocarcinoma. Measuring hCG and βhCG in postmortem serum and other nonblood fluid samples, in some pregnancy- and tumor- related fatal cases that underwent medico-legal autopsies, reveals the interesting role of hCG as a forensic biomarker.

Keywords: human chorionic gonadotropin, forensic, biomarker.

INTRODUCTION

The human chorionic gonadotropin (hCG) has two subunits (noncovalently linked), βhCG containing 145 amino acids and αhCG containing 92 amino acids, the α subunit being present in all glycoprotein hormones (follicle-stimulating hormone FSH, luteinizing hormone LH, thyroid-stimulating hormone TSH). hCG is an important hormone synthesized during pregnancy by syncytiotrophoblast cells. In pregnancy, hyperglycosylated hCG exerts autocrine control implantation and placental growth. The hyperglycosylated βhCG and hCG free β-subunit (βhCG) are produced particularly in advanced malignancies [1].

Main clinical uses of hCG immunoassays in living patients include the diagnosis and monitoring of intrauterine pregnancy, the diagnosis of extrauterine pregnancy, screening for preeclampsia and Down syndrome, and the treatment of assisted reproduction by intracytoplasmic sperm injection or *in vitro* fertilization. The hCG determinations are also used for the diagnosis approach in case of gestational trophoblastic disease, including hydatidiform mole (partial or complete), choriocarcinoma and placental site trophoblastic tumor, and for gonadal/extragonadal germ cell tumors, including nonseminomatous germ cell tumors. Another application is the doping control in athletes which utilize hCG after use of testosterone and anabolic steroids, for stimulation of the testicular function [2-5].

Postmortem hCG were determined by several immunoassays in various specimens from women with intrauterine and ectopic pregnancies and patients with choriocarcinoma. There are no other detailed publications on hCG immunoassays in other forensic fields [4, 6].

The hCG occurs in circulation and other body fluids in different molecular forms, including βhCG. In clinical laboratories, immunoassays usually assess intact hCG, total hCG or total beta hCG (intact hCG + hCG free β-subunit), and hCG free β-subunit [7], some of these immunoassays can be used in different legal medicine scenarios involving pregnant women and patients with germ cell tumors [5].

The objective of this minireview is to discuss the interesting role of hCG as biomarker in postmortem serum and other nonblood fluid samples in pregnancy- and tumor-related fatal cases that underwent medico-legal autopsies.

*Correspondence to: Anca Angela Simionescu, "Filantropia" Clinical Hospital, Department of Obstetrics and Gynecology, "Carol Davila" University of Medicine and Pharmacy, Dionisie Lupu Street, no. 37, Sector 2, 050474, Bucharest, Romania, E-mail: asimion2002@yahoo.com
#All the authors have equally contributed.

hCG AS A FORENSIC PREGNANCY-ASSOCIATED BIOMARKER

In legal medicine, it was shown, more than thirty years ago, that hCG is a stable and useful diagnostic biomarker of pregnancy in the bloodstains. The β-subunit of hCG was detected using a commercial rapid enzyme immunoassay with good specificity and sensitivity, this being of particular interest in forensic applications for pregnancy diagnosis [8]. When different bloodstains are encountered in a forensic setting, it is feasible to differentiate those from a pregnant woman from others. hCG, human chorionic somatomammotropin or human placental lactogen (hPL), total estriol and progesterone in these stains may be assessed by immunoassay methods [9].

hCG values may be determined in femoral and cardiac blood, vitreous humor and bile in women deceased early after delivery or while pregnant using an enzyme linked fluorescent assay. This ELFA method combines a two-step sandwich enzyme immunoassay with fluorescent detection. The conjugate combines mouse monoclonal anti-hCG immunoglobulin with alkaline phosphatase. This enzyme catalyzes the degradation of the 4-methyl umbelliferyl phosphate substrate into the fluorescent compound 4-methyl-umbelliferone, assessed at 450 nm [4].

Blood, vitreous humor and bile samples are analyzed by procedures performed similar to plasma and serum obtained from alive persons. The test is positive if the hCG level is greater than 4 IU/L. The lower detection limit is 2 IU/L. If hCG concentration is above 1600 IU/L, dilution have to be performed. All pregnant women samples are positive for hCG in all blood, bile, and vitreous humor autopsy samples. Concentrations are inferior in vitreous humor compared to blood, without correlation. This postmortem immunoassay has specificity indicated by the absence of false positives. It is adequate to determine uncertain pregnancies and to distinguish further circumstances in which the levels of hCG are raised. More data are needed to accurately assess the stability in postmortem specimens of the hCG, and to evaluate the sensitivity versus blood antemortem testing [4].

The sensitivity with this legal medicine approach limits its practical application when only minute dried bloodstains are investigated. Because the forensic diagnosis of pregnancy using bloodstains may be used in cases of criminal abortions and infanticide, RT-PCR (reverse transcription polymerase chain reaction) may be performed to detect βhCG mRNA transcripts from small bloodstains, involving whole blood, ethidium bromide staining and agarose gel electrophoresis. Although the βhCG mRNA cannot be measured all through the full period of a normal pregnancy, a combined analysis of pregnancy-specific human placental lactogen and βhCG mRNA biomarkers may estimate the gestational age of a woman from blood stains present at the forensic setting. It is considered that further developments of the RT-PCR βhCG assay are needed, including the extraction of RNA, the synthesis of cDNA, and the PCR amplification [10].

hCG AS A FORENSIC ECTOPIC PREGNANCY BIOMARKER

In the forensic field, several autopsy reports on maternal deaths associated with ruptured ectopic tubal pregnancies and with massive hemoperitoneum were reported. In such cases, biological samples are collected for hCG analysis shortly after the cadaver is transported to the morgue (in case of vitreous humor) and at the time of postmortem examination (serum, bile, cardiac and cerebrospinal fluids)[6]. In special circumstances, serum samples may be obtained from a deeply frozen deceased person after a postponement of about 40 h to permit the corpse to thaw, consequently being possible to take blood from the femoral vein [11].

Postmortem femoral blood specimens are obtained from the femoral veins without delay previous to autopsy. After clamping the vein at the proximal end with lifting the lower limb, blood is collected by aspiration via a syringe with sterile needle. Samples are then kept in special tubes with gel serum separator [6]. The undiluted left and right eye vitreous humor samples are collected by aspiration via scleral puncture (at lateral canthus), from the center of each eye. The undiluted specimens of cerebrospinal fluid are obtained via aspiration by suboccipital puncture, while pericardial fluid ones are collected straight away post pericardium incision. Undiluted bile specimens may be obtained by puncture from the gallbladder, and stored in preservative-free tubes [6].

After collection, the postmortem blood, vitreous, cerebrospinal and pericardial fluid samples are centrifuged for 15 min at 3000 g, without delay. After this step, the supernatant from vitreous humor, cerebrospinal fluid and pericardial fluid samples are stored in special tubes without preservative, and transported to the laboratory. If the immunoassays are not performed immediately, the specimens are frozen at -20°C [6].

The quantitative electrochemiluminescence immunoassay (ECLIA) with a sandwich format may be used to determine hCG. The samples are incubated with biotinylated hCG-specific monoclonal antibodies and hCG-specific monoclonal antibodies labeled with tris-bipyridyl-ruthenium-complex [Ru(bpy)3]2+. After adding microparticles coated with streptavidin in a second incubation, this complex is bound by streptavidin-biotin ligations to the solid phase. The microparticles are captured magnetically using an electrode into the measuring cell where the mixture is aspirated. A chemiluminescent emission is induced by an application of a voltage, after the unbound compounds are eliminated. A photomultiplier measures the emission and converts the results using a calibration curve [1,12,13].

hCG values of less than 5 IU/L are considered negative for pregnancy, considering the clinical reference levels actually applied for plasma and serum from alive patients. The postmortem values of total hCG in serum, vitreous humor, bile, cerebrospinal and pericardial fluids from ectopic pregnancies range from 25 to 55000 IU/L. The levels lower than 1 IU/L are usually found in postmortem specimens from non-pregnant cases [6].

Assuming that the levels in postmortem serum can be consistently compared to blood antemortem values, and considering that no false-negative postmortem results are expected, it is suggested that the ruptured ectopic pregnancy diagnosis may be considered in case of high hCG values in serum, pericardial, bile, vitreous humor or cerebrospinal fluid samples, but gestational age cannot be reliably estimated[6].

Even if no correlation is expected among hCG concentrations obtained in different types of specimens, measurements in nonblood biological fluids up to 48 h after death could be helpful in cases of blood unavailability subsequent to exsanguination or attempted massive lifesaving transfusion. Further investigations are necessary to better assess the postmortem stability and behavior of hCG in different specimens related to the period of time after death [6].

hCG AS A FORENSIC TUMOR BIOMARKER

hCG represents a sensitive biomarker for trophoblastic tumors and a useful biomarker for testicular germ cell tumors. Nearly all trophoblastic tumors secrete hCG. Although they also produce hCG free β-subunit, its concentrations are generally lower compared to hCG, with the exception of more aggressive forms associated with a higher proportion of βhCG. Testicular germ cell tumors are of either germ cell nonseminomatous tumors or seminomas. The nonseminomatous forms are categorized as choriocarcinomas, yolk sac tumors, embryonal carcinomas, and teratomas (mature or immature), many being mixed (consisting of different histological components). Approximately 50-70% secrete hCG, α-fetoprotein (AFP) or both. An increased βhCG serum concentration is detected in 50% of the patients with seminoma, the hCG presenting high levels in these cases (less than 20%) [2, 5, 14].

Postmortem hCG may be documented in specimens obtained from cases with choriocarcinoma. In this oncopathology, blood hCG values are expected to be comparable antemortem and postmortem[4].

The serum and urine concentrations of hCG may be significantly increased in the case of germ cell tumors, extragonadal and gonadal. High postmortem hCG serum levels may be detected in mediastinal neoplasms derived from germ cells, frequently presenting as a mixture of different histological types, such as choriocarcinoma and teratoma. Choriocarcinoma is described by a dual cell population composed of syncytiotrophoblastic cells and cytotrophoblasts. Because germ cell tumors are commonly located in the gonads, it is essential to rule out metastasis from an occult gonadal neoplasm before establishing a diagnosis of primary mediastinal tumor [15].

Tissue expression of hCG, detected in syncytiotrophoblasts, associated with high serum levels (above 300-1000 IU/L) occurs almost exclusively in nonseminomatous germ cell tumors [2,16].

Klinefelter syndrome patients are predisposed to germ cell tumors in the mediastinum, therefore karyotyping is useful [15].

Extremely high hCG levels (above 1500000 IU/L) were detected postmortem, together with normal AFP and carcinoembryonic antigen values, in a young male adult with primary mixed choriocarcinoma and mature teratoma found in the thymic region [15].

Choriocarcinomas generally appear in the ovaries and uterus, and rare extragenital choriocarcinomas in males usually develop in mediastinum or retroperitoneum. The autopsy of an old male patient with a very rare primary choriocarcinoma in the urinary bladder, first evaluated as transitional cell carcinoma, revealed a strong positive immunohistochemical staining for βhCG in the

cytoplasm of the syncytiotrophoblasts[17].

In the case of a gastric choriocarcinoma with positive β-subunit of hCG immunohistochemical staining revealed as cytoplasmic granular diffuse deposits in trophoblasts and syncytiotrophoblasts, high hCG levels were reported in the serum and urine autopsy samples[18].

In conclusion, hCG and βhCG in postmortem serum samples and other nonblood fluid specimens may be measured in particular pregnancy- and tumor- related fatal cases that underwent medico-legal investigations. This up-to-date short overview, dealing with various types of forensic samples and methods, reveals the interesting role of hCG as a forensic biomarker.

Conflict of interest

The authors declare that they have no conflict of interest.

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