

Human Chorionic Gonadotropin: "The Magic Molecule"

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Abstract

hCG is a glycoprotein molecule composed of two dissimilar subunits, alpha(α) and beta(β) subunits, linked together with hydrogen and disulphide bonds. All cells produce hCG. However hCG molecule secreted by the placenta has a longer half life, owing to the glycosylation of its β -subunit. The only definitely known function of hCG, is to support the corpus luteum of pregnancy. Uses of hCG include diagnosis of normal as well as nonviable, ectopic and molar pregnancy, as a tumor marker and for aneuploidy screening, while for therapy it is used in various steps of assisted conception, recurrent or threatened abortion, preterm labor and as antifertility vaccine. Many more areas are yet to be explored, where this enigmatic molecule has the capacity to do wonders.

INTRODUCTION

Human chorionic gonadotropin (hCG) derives its name from the fact that it is biologically similar to gonadotropins secreted by the pituitary gland (Follicular Stimulating Hormone or FSH and Luteinizing Hormone or LH), is produced by chorionic tissue and is the first hormone demonstrated in human species prior to its recognition in animals. ¹

HISTORICAL BACKGROUND

Story of hCG dates back to 1530 BC, as described in Berlin Papyrus. ² In Egypt pregnancy test consisted of moistening two cloth bags one filled with barley and the other with wheat grains with women's urine. Sprouting meant pregnancy test is positive. When wheat sprouted it was considered a baby girl while only barley meant a male conceptus. A study done in 1963 to determine the accuracy of this ancient pregnancy test surprisingly revealed a 70% rate of it being right.

It took more than 3000 years for the first pregnancy test based on hCG to be developed, initiating a new era in pregnancy testing. In 1927 Selmar Aschheim and Bernhard Zondek described A-Z test, which identified the presence of hCG in urine. ³ To test for pregnancy, a woman's urine was injected into an immature rat. In case of pregnancy, the rat would have estrous reaction and "blut punkte" (blood points) in the ovary, determined by a laparotomy approximately 100 h after the injection. Aschheim and Zondek had named this hormone – "prolan", derived from the Latin word proles,

meaning offspring. During early studies of the A-Z test, the scientists discovered that testicular tumors could also produce hCG. The 1930s saw a frenzied increase in shooting up all sorts of poor little creatures with hCG may it be a rabbit, frog, or a toad. These tests were expensive, required sacrifice of animals, and were slow, often taking days to get results.

Three decades later, in 1960, hemagglutination inhibition test, for pregnancy was developed by Wide and Gemzell. ⁴ This test was an immunoassay rather than a bioassay, was much faster and cheaper, but still relatively insensitive, especially for early diagnosis of pregnancy.

During 1970-1972 scientists at National Institute of Health (NIH) learned more about the properties of hCG. Using various methods, they identified two subunits of hCG. They found that the beta-subunit is where the immunologic and biologic specificity of hCG molecule resides.

FDA approval was sought in the year 1976 by Warner Chilcott for "Early Pregnancy Test (EPT)" later known as the "Error Proof Test" the first home pregnancy test kit in the United States.

After this first successful utilization, the magic molecule of hCG is trying to spread its pseudopodia in each and every territory of medical sciences, be it the sphinx of infertility, aging, obesity, or cancer.

BIOCHEMISTRY OF HCG MOLECULE

Though the isolation and identification of hCG was performed as early as 1960s, the crystallographic structure was first determined by Laphorn et al. in 1994. ⁵ hCG is a 40,000 dalton glycoprotein molecule composed of two dissimilar subunits, alpha(α) and beta(β) subunits, linked together with hydrogen and disulphide bonds.

The α subunit of hCG is similar to other glycoprotein hormones FSH, LH and TSH (thyroid stimulating hormone), but the β subunit is different in the sense that the terminal 28 to 30 amino acids of the carboxyl terminus are not found in any other glycoprotein hormone.

All cells produce hCG. However hCG molecule secreted by the placenta has a longer half life, owing to the glycosylation of β subunit, a unique function of chorionic tissue, that prevents its degradation. ⁴

HCG AND ITS ISOFORMS

hCG in the biological fluids, is not a single molecule, rather a mixture of different isoforms. Isoforms mean intact hCG, free subunits and degraded forms of these. International Federation of Clinical Chemistry (IFCC) established a working group in 1995 with the aim of improving standardization of hCG determinations. The nomenclature adopted by IFCC includes hCG, its subunits hCG α and hCG β , partially degraded or nicked forms of hCG (hCG $_n$) and hCG α (hCG α_n), and beta-core fragment (hCG β cf). ⁶ In addition to these variants, recently described hyperglycosylated isoform is of potential clinical utility. Produced by invasive cytotrophoblasts, it is the predominant form in second and the third week of pregnancy, slowly replaced by regular hCG. Specific measurement of this hyperglycosylated isoform is useful in suspected invasive gestational trophoblastic disease.

Point to be appreciated is that the clinical significance of these isoforms is not for routine diagnosis and follow up of normal pregnancy but for the follow up of gestational trophoblastic tumors, and germ cell tumors. Moreover various commercial kits manufactured to measure hCG have different specificity to different isoforms.

FUNCTIONS OF HCG

The only definitely known function of hCG to date, is to support the corpus luteum of pregnancy, ⁷ allowing continued progesterone production and maintenance of the gestational endometrium.

It might stimulate steroidogenesis in early fetal testis, ensuing androgen production and masculine differentiation. ⁸ This hypothesis is supported by the fact that in anencephalic fetus where the pituitary is absent, external genitalia are well developed in a male fetus, possibly owing to the presence of hCG. hCG gene is expressed in fetal kidney and adrenal glands, suggesting that hCG may affect the development and function of these organs. ⁹ In addition hCG may regulate placental development by influencing cytotrophoblast differentiation. ¹⁰

LABORATORY TEST FOR HCG

Qualitative tests: These are performed in urine and most of the times for detection of pregnancy. All hCG pregnancy tests are "sandwich" assays. Sandwich assay means there are two antibodies capture antibody and tracer antibody, in between these two, antigen that is the hCG molecule gets sandwiched. Tracer antibody refers to an antibody with a tracer that can be a dye, a radioactive material, a chemiluminescence agent or any other identifier.

Commercially available pregnancy test kit consists of a disposable plastic device which has three main parts – a well to pour drops of urine, another opening or window to see test result, and in between a column shielded by plastic this contains tracer in liquid phase antibody. If hCG is present in the urine poured into the well it will move towards tracer antibody in liquid phase and will bind to it, will continue to flow towards solid phase capture antibody present in a nitrocellulose membrane in the window, immobilized in a line shape, which glows with color if the poured urine contains hCG.

Quantitative test: Serum samples are preferred for quantitative hCG determination. Presently, virtually all commercial assays are the sandwich immunometric assays. ¹¹ Most serum assays are designed to measure both intact hCG and hCG α together.

DIAGNOSTIC DILEMMAS

Phantom hCG: Elevated hCG levels in absence of pregnancy, around 42% of times are because of phantom hCG. This is because of some heterophilic antibodies present in serum. A large number of patients have been and are still being needlessly treated with chemotherapy or hysterectomy based on phantom hCG levels. ¹² This false positive result can easily be ruled out by urine test, as the heterophilic antibodies do not cross the glomerular membrane.

Pituitary hCG: A positive hCG test during reproductive

years is an indicator of pregnancy. A positive hCG test in peri and post menopausal women poses a diagnostic challenge. Around three decades back in 1976 Chen et al, discovered pituitary hCG. ¹³ With age when pituitary starts failing, GnRH production loses the steroidal feedback control. As a result continuous GnRH stimulation of gonadotrops occur. Under these hyperstimulation conditions, pituitary at times starts secreting hCG like molecule. This hypothesis has been supported by extraction of hCG from pituitary extracts ¹⁴, secretion of hCG by cultured fetal pituitary cells, ¹⁵ identification of hCG secreting cells in anterior pituitary, ¹⁶ and identification of β hCG mRNA in the pituitary gland. ¹⁷ The discovery that hCG of pituitary origin can be suppressed by estrogen and progesterone by Stenman et al in 1987, led to a valuable tool for differentiating pituitary hCG. ¹⁸

DIAGNOSTIC USES OF HCG

Normal pregnancy: hCG-mRNA is detectable in the blastomeres of six to eight cell embryos. However its estimation in the maternal circulation is possible only after implantation and establishment of feto-maternal circulation, on day 21 after last menstrual period. During early pregnancy, hCG concentration in serum increases exponentially doubling on an average every 1.5 to 2 days. Maximum concentrations ranging from 20,000 to 100,000 IU/L are reached at 8-10 weeks. After this the levels start falling, plateauing out at 13-15 weeks and maintain a low level till term.

Nonviable pregnancy: Serial quantitative assessment of serum hCG serves as a biomarker of pregnancy outcome. In a normal pregnancy, first trimester hCG concentration rapidly increases, doubling every two days. Traditionally 66% rise has been used as a cut off for viability. ¹⁹ Though recent evidence suggests that 53% increase in hCG concentration every two days should be considered as the limit for viability. ²⁰

Ectopic pregnancy: Ectopic pregnancy is diagnosed by high index of suspicion.

Neither single nor serial measurements can confirm the location of gestational sac. Ectopic pregnancy may present with rising, falling or plateau hCG levels. Serial measurement is useful to confirm viability rather to identify ectopic pregnancy. Demonstration of normal doubling over 48 h supports diagnosis of fetal viability, but does not rule out ectopic pregnancy. Similarly, falling level confirms non-

viability, but does not rule out ectopic pregnancy. ²¹

Still in ectopic pregnancy hCG is helpful in conjunction with ultrasound, as well as to guide methotrexate treatment and its follow up.

Molar pregnancy: For the diagnosis of molar pregnancy ultrasound alone is adequate, quick and safe. Serial hCG assays are limited for follow up. Choriocarcinoma commonly occurs after molar pregnancy, though it might develop after ectopic pregnancy or normal pregnancy too. For choriocarcinoma, the sensitivity and specificity of hCG approaches 100%. Trophoblastic tumors containing only about 100,000 cells can cause elevated serum concentrations of hCG, which thus is by far the most sensitive tumor marker known. So this is the only malignancy where initiation of treatment is recommended on the basis of persisting, rising, plateauing hCG levels after molar evacuation, without any histopathological diagnosis.

Tumor marker: The role of hCG and its isoforms as tumor markers for gestational trophoblastic neoplasia and germ cell tumors is well established today. However many of the first studies on ectopic expression of hCG-like immunoreactivity in cancer were performed on cervical and ovarian cancer cell lines ^{22,23,24} Increased levels of hCG occur in urine of patients with cervical intraepithelial neoplasia (CIN) as well as in cervical, endometrial and ovarian cancers. High levels of hCG are reported to be associated with adverse outcome of cervical and vulvo-vaginal cancers. In ovarian cancer too a high hCG level has been shown to be a strong prognostic factor independent of stage and grade. ²⁵

Aneuploidy screening: The safest and most acceptable methods for prenatal screening of aneuploidy are maternal serum markers in conjunction with fetal ultrasound markers. In first trimester hCG can be used as an aneuploidy marker with pregnancy associated plasma protein A (PAPP-A), whereas in second trimester it is one among the components of triple or quadruple test with alpha fetoprotein, unconjugated estriol and inhibin-A.

THERAPEUTIC USES OF HCG

Assisted conception: In assisted reproductive techniques (ART), administration of partially purified urinary hCG preparation has been used for decades as a surrogate for LH to achieve final oocyte maturation and ovulation in controlled ovarian hyperstimulation (COH) protocols. The long half life of hCG ($t_{1/2} = 24$ h) makes it a better alternative than LH ($t_{1/2} = 2$ h) facilitating timing of oocyte retrieval in

connection with IVF/ICSI. It is also beneficial for in vitro maturation of follicles and as a component of culture system for oocyte maturation. ²⁶ hCG can also be used as an ovulation trigger in assisted conception with ovulation induction, timed intercourse or intrauterine insemination (IUI).

Recently, hCG produced by recombinant techniques in Chinese hamster ovary cells has become commercially available. In ART, a dose of 250 µg of rhCG has been found to be equivalent ²⁷ or at least as effective as 10,000 IU of urinary hCG in inducing final stages of oocyte maturation. Furthermore, the use of rhCG was associated with significantly better patient tolerance. ²⁸

Recurrent and threatened abortions: Though hCG is being used for the management of recurrent abortion, evidence does not support this. The role of hCG for threatened abortion falls into a grey area, there are studies that have proved that hCG has an ultrasonologically, clinically, and biologically proven effect on viable trophoblasts in cases of threatened abortion. ²⁹

A recent study, published in 2008, found no difference in mean gestational age at delivery, birth weight, and preterm birth, concluding that even if there is not much evidence to support the use of hCG in threatened or recurrent abortions, there is no harm too, in giving the benefit of doubt as hCG treatment in early gestation does not increase chances of adverse effect in neonates. ³⁰

Preterm labor: Data suggests that hCG may play a role in maintenance of later stages of pregnancy as well, by directly and indirectly promoting uterine quiescence. ³⁴

Antifertility vaccine: hCG vaccine is the world's first family planning vaccine, developed by Dr Gurusaran Talwar in 1976, based on the fact that hCG is necessary for maintenance of pregnancy and neutralization of hCG activity with antibodies can be used for contraception. However as extensive clinical studies have not been performed, contraceptive vaccines.

HCG IN SPECIAL SITUATIONS

Ovarian Hyperstimulation Syndrome (OHSS): It is a rare but potentially fatal complication of ART. The initializing event is increased production of vascular endothelial growth factor (VEGF), increasing vascular permeability leading OHSS, which may vary from mild abdominal discomfort to ascitis, pleural effusion, fluid and electrolyte imbalance, renal

failure and in rare instances even disseminated intravascular coagulation (DIC). High level of hCG causes increased production of VEGF and is considered as the root cause of OHSS.

Hyperemesis gravidarum: There are a number of reasons for considering the association of hCG with hyperemesis gravidarum. The temporal relationship between peak hCG levels and the most common time of nausea and vomiting has long been noted. Moreover, several experiments of nature have suggested that hCG plays a role. Nausea and vomiting of pregnancy (NVP) is more common in women with molar and multiple gestation in which hCG is significantly elevated. More recently, it has been noted that mothers carrying fetuses with Down syndrome, a condition associated with elevated hCG, are more likely to have NVP.

THOUGHTS TO PONDER

In early pregnancy, vertical transmission of HIV is extremely rare due to high concentration of hCG, as the β -subunit is active against HIV. By the second trimester women carrying female fetus have higher hCG levels than those carrying male fetus. hCG acts as an endogenous tocolytic in normal pregnancy. It may provide a promising pharmacological approach to the pervasive problem of preterm labor. Qualitative hCG testing of cervico-vaginal washings appears to be useful predictor of preterm rupture of membranes.

A double-blind, placebo-controlled, randomized clinical trial of rhCG done by Liu PY et al. showed beneficial effects on muscle strength, physical function as well as activity in older men with partial age-related androgen deficiency.

In 1954, Dr. Simeons, a British-physician claimed hCG injections could suppress appetite, burn stored fat and redistribute fat. Unfortunately, he had no clinical evidence to validate his claims. Since then a number of studies have been done quite extensively. However, not a single study indicates hCG has any benefit for weight loss. Though this use of hCG has not been approved by FDA, weight reduction clinics are using it with immense patient satisfaction.

hCG, is found, curiously enough at the surface of all cancer cells. Connection between cancer and pregnancy was first muted by Dr Cohnheim. Many scientific studies have correlated increased β -hCG levels with increased risk of malignancy and metastasis. Studies have shown hCG inhibits Kaposi sarcoma associated angiogenesis and tumor

growth. It is also being postulated to be helpful in the prevention of breast cancer.

CONCLUSION

hCG is a glycoprotein hormone with unique β -subunit. Its main function is to support corpus luteum. It has many diagnostic and therapeutic benefits. Many more areas are yet to be explored, where this enigmatic molecule has the capacity to do wonders.

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