Human Chorionic Gonadotropin

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4.1 Introduction

HCG is a hormone unique to gestation, however, it may be raised in other pathological states as well. Human chorionic gonadotropin (hCG) is a placental hormone secreted after implantation and is commonly detected by urine gravindex test. It interacts with the LHCG receptor of the ovary and maintains the corpus luteum during initial weeks of pregnancy. It is also produced by most of the trophoblastic tumors where the serial quantitative detection with rise and fall gives information about the course of the disease, prognosis, treatment, treatment response, and recurrence. Various levels of rise are observed in gestational trophoblastic disease and very very high level in its malignant counterpart Gestational Trophoblastic Neoplasia, including Choriocarcinoma. It is secreted mainly by the syncytiotrophoblasts soon after implantation, though also secreted in small amounts by the anterior pituitary. hCG is solely responsible for maintaining pregnancy before progesterone takes over at 12 weeks of gestation. There are various different forms of hCG that have been identified, although the function of each type is yet to be defined. It is imperative that whatever information is available be utilized for the accurate characterization of hCG-associated lesions [1].

This chapter would focus on the different types of hCG and their biological significance.

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4.2 Structure

HCG is composed of two subunits alpha (α) and beta (β) held together by non-covalent hydrophobic and ionic bonds and contain a total of eight carbohydrate side chains [1]. It is composed of 237 amino acids (92 in α and 145 in β) and the molecular weight is approximately 36 KDa. The alpha-subunit is homologous to that of TSH, LH, and FSH whereas the β-subunit has 80–85% homology to LH. The difference between β-subunit of LH and hCG is in 24 amino acids in the carboxyl-terminal peptide (CTP), which is unique to hCG. Consequent to this sharing, there is cross-reactivity between the two molecules; also the longer half-life of hCG compared to LH is attributed to the four glycosylated serine residues in the CTP. Thirty percent of the weight of hCG is due to the eight carbohydrate side chains. It is the changes in these side chains, which result in three different types of hCG isoforms: regular hCG, sulfated hCG, and hyperglycosylated hCG [2, 3]. The alpha-subunit is encoded by a single gene at chromosome 6q12–q21 while the β-subunit is encoded by six non-allelic genes located at chromosome 19q13.32 [4].

4.3 Production

Naturally, it is produced in the human placenta by the syncytiotrophoblast. hCG peaks at 10 weeks of gestation then plateaus as gestation advances. So, it is likely to be raised wherever syncytiotrophoblast is present, for example—gestational trophoblastic neoplasia and germ cell tumors with trophoblastic elements. In consensus with the site of production, 15–20% of seminiferous testicular tumors and 40–50% non-seminiferous testicular tumors secrete hCG. Traditionally for pharmacological use, it has been extracted from the urine of pregnant woman, but a more purified form can be produced using genetically modified techniques, which is free from any contamination and is thus, much safer to use.

4.4 Metabolism

It is primarily metabolized by the liver and 20% is excreted by the kidneys. The half-life of injectable HCG and that produced during pregnancy differs slightly as the purified injected forms get partly denatured during processing. Also, there are differences in clearance rates of the alpha and β-subunit with the latter having a longer half-life owing to differences in glycosylation. The major byproduct of hCG metabolism is the β-core fragment (cf). More than 99% of HCGβcf is formed in the kidneys during renal excretion. After injection of urinary HCG, HCGβ, or recombinant HCG (rHCG), peak concentrations of HCGβcf occur approximately 6 h after the HCG peak in urine [5, 6].

4.5 Functions

- Immunomodulation—hCG protects the developing fetus from the immune system of the mother [7, 8] hCG has a high negative charge, so repels the immune cells of the mother, protecting the fetus during the first trimester. hCG is a link in the development of peritrophoblastic immune tolerance, which facilitates the trophoblast invasion responsible for development of suitable environment in endometrium.
- Implantation—helps in trophoblastic invasion**.**
- Early pregnancy—hCG binds to its receptor of the ovary and maintains the corpus luteum up to 12 weeks till the placenta takes over. It has also been suggested that hCG levels are linked to the severity of morning sickness or Hyperemesis gravidarum in pregnant women.
- Ovulation induction—Because of its similarity to LH, hCG is used clinically to trigger ovulation in ART procedures.
- Male pseudo-hermaphroditism—to increase testosterone production.

4.6 Forms of hCG

Various isoforms of hCG are known like total hCG, intact hCG, free β-subunit hCG, hyperglycosylated hCG, nicked hCG, alpha hCG, and pituitary hCG [2, 3].

Only the intact, hyperglycosylated, and pituitary variants have been described in detail in the following text.

4.6.1 Intact hCG

This is the main form of hCG associated with the majority of pregnancy and in noninvasive molar pregnancies. This is produced in the trophoblast cells of the placental tissue.

4.6.2 Free β-subunit hCG

The difference between free β-subunit and β-subunit is that the free β-subunit is a hyperglycosylated variant of the β-subunit of hCG. Increased levels are seen in hydatidiform mole, choriocarcinoma, and non-trophoblastic cancers of all primaries. It has an important role to play in non-gestational neoplasm by serving as a promoter of malignant transformation thereby leading to poor clinical outcome [6]. Hence, targeted antibody directed to this specific subunit can help in controlling the disease. Owing to this knowledge, efforts are ongoing to develop a vaccine containing β-subunit for treating non-gestational malignancies.

4.6.3 Hyperglycosylated hCG

Glycosylation of hCG occurs by any sequential addition of carbohydrate side chains just before release of the assembled dimmer, it is not only of structural importance but has an important functional role as well. Glycosylated hCG has a different rate of clearance from the body apart from a unique biological action. This process of glycosylation of hCG is variable and this leads to the production of a variety of glycoforms and only some have been understood till now. The glycosylation of tumor-derived hCG is also highly variable with increased amount of abnormal glycans and hence referred to as hyperglycosylated. This hyperglycosylated hCG is recognized by a specific antibody, B152. This form of hCG could serve as a biomarker of an invasive trophoblastic phenotype [9].

4.6.4 Pituitary hCG

Pituitary hCG or sulfated hCG is another form secreted by the pituitary gland. It contains half of sialic acid as that of intact hCG and some of the glycans are sulfated and hence the name. It is relatively less active than hCG. Its concentration increases during menopause and is about 0.5–5 IU/L for most women. Levels have also been found to be raised post chemotherapy. Although it is increased at low levels only, but it is important to differentiate from early pregnancy or malignant disease.

4.7 Clinical Importance of Different Isoforms

4.7.1 Pregnancy

Intact HCG comprises more than 95% of total hCG in maternal circulation during the few weeks of pregnancy. The free alpha-subunit also comprises less than 10% in the first trimester but increases throughout pregnancy to become 30–60% at term. The free β-subunit is less than 10% in very early pregnancy and is 0.5–2% after the eighth week. Urinary concentrations of free β-subunit are, however, higher (9–40%) [10]. Hyperglycosylated hCG is the predominant form of hCG in early pregnancy in serum and urine. Low levels are seen in preeclampsia and associated with pregnancy loss [11, 12]. It has also been considered as a marker of normally functioning invasive extra villous cytotrophoblast necessary for implantation. Hence, ineffective invasion is usually seen due to insufficient hyperglycosylated hCG. High levels seen in Down syndrome are used as a screening method in high-risk pregnancy [13].

The most important form in urine is composed of hCG β-subunit core fragment hCG. It is the final product of hCG metabolism. However, it is not recognized by the many available commercially available hCG assays. Nicked forms are more rapidly cleared than intact heterodimer and moreover, due to their relatively higher concentrations, they are more abundant in urine than serum. The proportion of the nicked

form increases during pregnancy, such that the ratio of nicked to intact in urine becomes 31 from 8. This particular isoform gets elevated with preeclampsia and Down syndrome [2, 3].

4.7.2 Tumor

Hyperglycosylated form is the principal hCG secreted by choriocarcinoma. It not only promotes cytotrophoblast invasion in a normal pregnancy but also mediates invasion in choriocarcinoma. Increased levels of the free β-subunit of hCG have been identified in almost all tumors within the Trophoblastic neoplasia spectra. The free β-subunit of hCG does not lead to activation of the LH receptor but has growth-promoting activity. Placental site trophoblastic tumor has the highest proportion of the free β-subunit among all other types [2, 3]. The nicked forms are the major form of hCG following molar evacuation apart from being increased in testicular and bladder cancer. Even in these patients, the hCG β-subunit core fragmentations are the predominant urinary form. But, the free β-subunit has been recognized as a better marker in non-trophoblastic tumors.

4.8 Testing

A number of tests are available to detect regular hCG and β hCG specifically, but it is difficult to measure other forms of hCG. Many hCG immunoassays are based on the sandwich principle, which uses antibodies to hCG labelled with an enzyme or a conventional or luminescent dye [14]. Antibodies employed are of two types—a solid phase antibody to capture hCG molecules from the sample—capture antibody and a second one labeled with an enzyme or a conventional or luminescent dye—detector antibody [15]. International reference reagents for other important forms of hCG have been formulated including—nicked hCG, free alpha-subunit of hCG, nicked free β-subunit of hCG, and hCG β-subunit core fragment. However, no international standard for hyperglycosylated hCG is available for use at present because of the variable carbohydrate composition of this isoform. One important point to note here is that the most common commercially available test kits measure total hCG and not just β-hCG as they are commonly referred to. Detection of not total or intact hCG but these isoforms is more useful in the oncology setting. Oncologists are more concerned with respect to the free β-subunit, nicked and fragment forms of free β-subunit than the intact hCG as it helps them predict the disease course to an extent.

4.8.1 False Results

Analytical errors are uncommon with immunoassays being used but can have serious adverse consequences when neoplasia is suspected. However, they can be easily

overcome when analyzing along with the clinical picture. Errors due to the specimen itself are particularly hard to detect due to sporadic presence within the serum of cross-reacting substances interacting with the assay's antibodies or to the target itself.

4.8.1.1 False Positives

Heterophilic Antibodies
These are naturally occurring antibodies with low affinity to many antigens and are responsible for a false-positive result. Examples of such antibodies are Rheumatoid Factor (autoantibodies found in 5–10% of the general population and 70% of Rheumatoid Arthritis patients). These heterophilic antibodies are the most common cause of false positivity of hCG assays [16].

Phantom hCG
First described by Laurence Cole, in 1998, when he observed persistent mild elevations of hCG in women following miscarriage [17]. This elevated hCG often translated into treating patients with cytotoxic agents for persistent disease when in reality no hCG or trophoblastic disease is present. It is a consequence of the interference of heterophil antibodies with standard assays for hCG. However, in recent years, different hCG assays have been marketed, which vary in their response to measurement of human anti-animal antibodies and heterophil antibodies which has decreased the incidence of this phenomenon [1]. The American College of Obstetrician and Gynecologists recommends three procedures to rule out the presence of heterophil antibodies. The first is the urine test; if urine is negative for hCG and the serum value is at least 50 IU/L then interpretation of interference can be made. The second method is by serial dilutions, nonlinearity suggests the presence of this interference. Lastly, pretreating the serum to remove heterophil antibodies may be used. All of the above counteractive measures will help to alleviate the interference by "phantom" hCG and minimize unwarranted investigations and therapeutic interventions in individuals with suspected pregnancy or trophoblastic diseases [18]. The problem of "phantom" hCG has been identified since the early 1970s when classic competitive immunoassays were in use [19].

hCG Injection
Exogenously administered hCG can lead to false positivity of a sample. hCG has been used as an alternative to LH for controlled ovarian hyperstimulation in ART, levels as high as 60–300 mIU/ml are reached but are completely cleared within 2 weeks of administration. hCG injections have also been misused to stimulate gonadal steroid production by athletes.

Anti-hCG Antibodies
These are an unlikely source of error, but common in women who have received hCG injections for infertility treatment.

Familial hCG

It is a rare genetic condition in which both serum and urine hCG concentrations are persistently elevated for several years. The individual is asymptomatic and thus recognition is important to avoid unnecessary treatment. The concentrations are usually low 10–200 mIU/ml and it should be considered after other conditions have been ruled out. Presence of similar findings in first-degree relatives can confirm the diagnosis. Till now, this condition has been reported in ten families only [20].

Quiescent hCG Syndrome

A small group of patients have a prolonged but less rise of β hCG with a double-digit titer and persists for few long years in absence of radiological evidence. It may be due to small foci of dispersed and differentiated syncytiotrophoblast. These slow growing trophoblastic cells make persistent slow rise of hCG but do not possess power of invasiveness nor do these cells respond to chemotherapy or hysterectomy. However, they could be a marker of early GTN [21].

4.8.1.2 False Negatives

Premature Measurement

Early measurement soon after conception, especially when the menstrual cycle is irregular can result in failure to identify the ongoing pregnancy.

Hook Effect

This is frequently observed in conditions where hCG values are very high, usually greater than 500,000 mIU/mL. This is seen when there are so many hCG molecules that they saturate the tracer and the antibodies separately, not allowing for the sandwiching of the tracer–hCG–antibody. Consequent to it, the complexes are washed away and not analyzed due to non-formation of tracer–hCG–antibody complex, giving a false-negative result. To avoid this phenomenon, predilution of the sample is necessary [22].

4.9 Conclusion

hCG is a glycoprotein hormone produced by a variety of organs in various gestational and nongestational events with diverse functions. The complete clinical relevance apart from diagnosis and management of pregnancy and pregnancy-related disorders to cancer surveillance is yet to be understood. Further research pertaining to its receptors, its different isoforms, and their functions are needed to be understood and its role in various clinical settings.

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