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Human Chorionic Gonadotropin (β HCG)-A Review

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ABSTRACT

The first trimester screening programme offers a noninvasive option for the early detection of aneuploidy pregnancies. This screening is done by a combination of two biochemical markers i.e. serum free b-human chorionic gonadotrophin (free β-hCG) and pregnancy associated plasma protein A (PAPP-A). The association between advancing maternal age and increased risk of trisomy 21 is well known, and pregnant women older than 35 years at delivery are routinely offered invasive prenatal diagnostic testing. The most commonly used test for genetic diagnosis is amniocentesis, but the rate of spontaneous fetal loss related to amniocentesis averages about one in every 200 procedures. Because of this risk, serum analyte testing has become an important, noninvasive first step in detecting patients at risk for congenital abnormalities. Screening for biochemical testing and ultrasound scanning can also be carried out in two separate visits, with the first done at 9–10 weeks and the second at 12 weeks .In screening for trisomy 21 by maternal age and serum free β-hCG and PAPP-A, the detection rate is about 65 % for a false-positive rate of 5 %. β-HCG is highly sensitive as a single serum measurement for the prediction of pregnancy outcome. Several of the recent observations based on these biochemical tests provide stimuli for the development of novel therapies for future application as mentioned above. High levels of β- hCG indicate poor prognosis and frequent assays during therapy level correlated to the clinical response. Serum HCG levels are rarely elevated in nontrophoblastic tumors such as lung, breast, pancreas and bladder cancers. When HCG levels plateau prematurely or fail to rise as expected, we consider that the pregnancy might not be viable. Normal HCG values vary up to 20 times between different pregnancies and an HCG that does not double every two to three days does not necessarily indicate a problem with the pregnancy. Some normal pregnancies will have quite low levels of HCG, and result in perfect babies.

KEYWORDS Human Chorionic Gonadotropin, Pregnancy, Down Syndrome, Malignancies

INTRODUCTION

HCG, A marker of germ cell tumors and trophoblastic disease, is 45KD glycoprotein, composed of two dissimilar subunits the alpha chain (14 KD) and beta chain (24KD). It contains 30 % carbohydrate. The beta subunit determines the immunological and hormone specificity. HCG is synthesized by the synctiotrophoblasts of the placenta during pregnancy. The peak HCG concentration is reached between 10th & 12th weeks of gestation. The reference values in serum of healthy men and non-pregnant women are less than 5 IU /ml and post-menopausal women are less than 10 IU /ml. HCG is a marker of first choice for gonadal (testes and ovary) choriocarcinoma and extragonadal choriocarcinoma. HCG shows 100 % sensitivity for choriocarcinoma irrespective of their site in addition to hydatidiform mole. In testicular tumors, the detection of HCG and AFP correlates with the histological findings, and is therefore crucial for the therapeutic procedures with the use of serial determination of β-hCG, the biochemical recurrence precedes by 3 months before the patient has symptoms of clinical recurrence / metastases. The marker also helps in monitoring high-risk group of testicular tumors especially individual with undescended testicle or the healthy monozygotic twin of a testicular tumor patient. High levels of β hCG indicate poor prognosis and frequent assays during therapy level correlated to the clinical response. Serum HCG levels are rarely elevated in nontrophoblastic tumors such as lung, breast, pancreas and bladder cancers.

The accuracy of your hCG urine test results will depend on your ability to closely follow the test kit's instructions. If you have a negative result, you should consider these results to be uncertain, as they may indicate a false negative. Until you can be sure that you're not pregnant, you should be cautious and avoid doing anything that could hurt a developing fetus. Smoking, using alcohol, and certain medications can harm your baby in early pregnancy.

A false-negative result can happen after any of the following:

- using a urine sample collected after your first morning urine
- taking the test before there's enough hCG to produce a positive result
- miscalculating the timing of your missed period

If you have a negative result, you should repeat the test in about a week to confirm the absence of pregnancy. If you believe the tests are indicating a false negative and that you're pregnant, you should consult your doctor. They can conduct an hCG blood test, which is more sensitive to lower levels of the hCG hormone than the hCG urine test.

The first pregnancy test based on the biological activity of human chorionic gonadotropin (hCG) in partially purified urine was described in 1927. Various modifications of this assay were used until the introduction of immunoassays in 1960[Wide L et al, 1960].With the advent of monoclonal antibodies, new specific assays for the various subunits and degradation products of hCG were developed. In 1984, the two-antibody immunometric assays for hCG were developed [Hussa RO et al, 1984]; during the same time period, antibody enzyme labeling and highly sensitive fluorimetric and chemiluminescent detection were developed — the basis of hCG tests used in all commercial labs today, and in point-of-care (POC) and over-the-counter (OTC) tests sold today. The introduction of efficient and sensitive hCG assays widened the application of hCG for diagnosis of pregnancy, trophoblastic disease, and non-trophoblastic neoplasms [Stenman UH et al, 2004]. It also led to the rise of a body of hCG research and the discovery of multiple hCG variants and their independent biological functions.

hCG biochemistry:

Maternal serum free β-hCG hormone Human chorionic gonadotropin is a 39,500-Da glycoprotein hormone normally

found in blood and urine only during pregnancy. In 1987, Bogart et al. reported an elevated levels of maternal serum hCG in Down's syndrome pregnancies, and since then hCG has been introduced in most screening programs. For the initiation and maintenance of pregnancy, hCG mediates multiple placental, uterine and fetal functions. Some of these include development of syncytiotrophoblast cells, mitotic growth and differentiation of the endometrium, localized suppression of the maternal immune system, modulation of uterine morphology and gene expression and coordination of intricate signal transduction between the endometrium [Banerjee P etal, 2011].

Chemistry:

Human chorionic gonadotropin hormone is composed of two noncovalently linked subunits, a and b, and is produced by syncytiotrophoblast cells of the placenta. hCG has a single bsubunit which contains 145 amino acids linked by six disulfide bridges and an a-subunit which contains 92 amino acids linked by 5 disulfide bridges, which is also shared by three other glycoprotein hormones: LH, FSH, and TSH. The b-subunit is unique, and distinguishes hCG from the other glycoprotein hormones. It contains two N-linked oligosaccharide side chains, attached to residues 13 and 30 [Ulf-Hakan S et al, 2006]. It also has four O-linked oligosaccharide units, located in the unique proline- and serine-rich C-terminal extension (residues 122–145). The a subunit has two N-linked oligosaccharide side chains, attached at amino acid residues 52 and 78 [Ulf-Hakan S et al, 2006]. Five hCG-related molecules are present in maternal serum: nonnicked hCG, which represents the active hormone; nicked hCG; free a-subunit; free b-subunit; and the nicked free b subunit [Trenti T et al, 2011]. The free b-subunit can derive from three sources, i.e., direct trophoblast cell production,dissociation of hCG into free a- and free b-subunits, and by macrophage or neutrophil enzymes nicking the hCG molecule [Fernando MR et al, 2006]. The free β-hCG circulating in maternal serum corresponds to only about 0.3–4 % of the total hCG [Fernando MR et al, 2006]. A single gene on chromosome 6 codes for the a subunit of all four glycoprotein hormones (TSH, LH, FSH and CG). Chromosome 19 contains a family of genes that encodes the CGb subunit [Fernando MR et al, 2006]. Separate messenger RNAs (mRNAs) are transcribed from each. The subunits spontaneously combine in the rough endoplasmic reticulum and are then continuously secreted into the maternal circulation.

Free β-hCG in Pregnancy Maternal serum hCG peaks at 8–10 weeks and then declines to reach a plateau at 18–20 weeks of gestation and remains quiet constant until term. β-hCG lacks hCG activity, but several lines of study indicate that it exerts growth promoting activity. It has been speculated that β-hCG interferes with the growth-inhibiting effect of transforming growth factor-b, platelet-derived growth factor-B and nerve growth factor [Butler SA et al, 2004]. The half-life of injected hCG is biphasic; the rapid phase has a half-life of 5–6 h whereas that of the slower phase is 24–33 h. The half-life of purified β-hCG injected into human is 0.7 and 10 h which is shorter than that of hCG. However, after term pregnancy or an abortion, β-hCG actually disappears more slowly than that of hCG. Thus the proportion of β-hCG of total hCG immunoreactivity increases from 0.8 % at term to 27 % after 3 weeks. A Molecular biology studies have demonstrated that trisomy 21 trophoblasts show a marked increase in β-hCG mRNA and a smaller increase in a-hCG mRNA, suggesting that one of the causes of high hCG levels in maternal serum is the increased hCG production and secretion by the placenta within short time of obtaining a blood sample makes it possible to combine biochemical and ultrasonographic testing for early assessment so that patients assessment and stress may be reduced. Separation of the sera for Down syndrome screening in 4 h after withdrawal is necessary.

Cooling during any storage, including transportation is highly recommended as the preanalytical phase has a high impact for the analysis. Serum samples for free β-hCG and PAPP-A are stable at 4°C for whole blood or separated serum for 1 week. Reliable results are obtained if separated serum samples are stored at 20° C up to 2 days and 1 day for whole blood. At 30°C

reliable results were obtained only if the samples were analyzed within 2 h collection. In whole blood, free β-hCG levels increased more rapidly compared to serum, especially at 30°C [Cowans NJ et al, 2010]. Several studies have reported that a high storage temperature and a long interval between collection and analysis of the sample produce an increase in the concentration of free β-hCG because it is liberated by the dissociation or degradation of intact hCG [Cruz J et al, 2010]. In whole blood kept at room temperature, the mean serum concentration of free β-hCG was reported to increase by 10–15 % after 24 h, by about 25 % after 3 days and by 45 % after 4 days [Cruz J et al, 2010]. Another study showed that PAPP-A levels are stable in serum for 142 days at 2–8_, 37 days at room temperature and 20 days at 30°C. There was no significant change with either analyte after-20°C storage for up to 240 days or after six repeated freeze-thaw cycles [Cowans NJ et al, 2010]. Presently, virtually all commercial assays are the enzyme amplified chemiluminescence technology. Maternal serum PAPP-A and free β-hCG can also be measured using a random access immunoassay analyzer using time-resolved amplified cryptate emission technology.

hCG belongs to the glycoprotein-hormone family, along with luteinizing hormone (LH), folilcle stimulating hormone (FSH), and thyroid stimulating hormone (TSH). It is a heterodimer composed of two subunits a and b, joined non-covalently. The a-subunit composed of 92 amino acids linked by five disulfide bridges is common to all members of the glycoprotein-hormone family. It has two N-linked oligosaccharide side chains at amino-acid residues 52 and 78. The b-subunit is unique to hCG and confers its biological activity. It is composed of 145 amino acids linked by six disulfide bridges and contains two N-linked oligosaccharides, four O-linked oligosaccharide chains, and a proline and serine-rich C-terminal extension (C-terminal polypeptide). hCG displays extensive charge heterogeneity due to variation in its scialic-acid content. Because of heterogeneity of carbohydrate moieties of hCG, the molecular weight displays a range of values. The average molecular weight of hCG is 37500 Da, with the a-subunit being 14000 Da and the b-subunit 23500 Da.

Multiple variants of hCG that exhibit independent functions are currently recognized. Hyperglycosylated hCG, which is an hCG variant with additional carbohydrate residues that is produced by cytotrophoblast cells [Kovalevskaya G et al, 2002], is the principal molecule produced in early pregnancy. The free b-subunit of hCG, which is made by non-trophoblastic neoplasma, is the principal molecule produced in cancer. Therefore, the term hCG refers to a group of variants that have the same a– and/or b-subunit peptide structure but have separate biological functions. Regular hCG functions in maintaining pregnancy much more consistently with its concentration during the length of gestation. It mediates its action through the LH/hCG receptor by maintaining progesterone production early during pregnancy. Hyper glycosylated hCG produced in invasive trophoblastic disease, choriocarcinoma, and in early pregnancy displays a different structure from that of regular hCG [Elliott MM et al, 2006]. It was shown to predominate in early pregnancy-serum samples, to be directly associated with the implantation of pregnancy, to be associated with invasion in invasive mole, and gestation-trophoblastic neoplasms including choriocarcinoma [Cole LA e tal, 2006]. In addition, the free b-subunit (-hCG) is produced by many non-trophoblastic neoplasms. It has been detected in the media of several malignant cell lines such as cervical, breast, bladder, ovarian, brain, colorectal, uterine, and lung [Cole LA e tal, 2007]. The free b-subunit is not, however, a reliable diagnostic marker for specific malignancies but, rather, has potential as a marker of poor prognosis [Cole LA e tal, 2007].

A variety of dissociation and degradation products of hCG b-subunit are detected in serum and urine samples. The action of the enzyme leukocyte elastase-like protease on hCG generates nicked hCG, nicked hyperglycosylated hCG, and nicked free b-subunit. These molecules are further degraded by the action of proteases,

including leukocyte elastase, via the cleavage of the b-subunit C-terminal segment (CTP; peptide resides 92-145), generating nicked hCG, nicked hyperglycosylated hCG and nicked free b-subunit missing the CTP. It is noteworthy that there are 10 common degradation variants of hCG detected in serum and/or urine samples. These are found in serum and urine samples in pregnancy, gestational-trophoblastic disease, and different maligncies. The variability in the forms of hCG found in various conditions must be considered when measuring total hCG in different stages of pregnancy and different cancer cases.

Uses of hCG testing:

Pregnancy: The only FDA-approved use of hCG testing is for pregnancy detection. hCG tests are also often used as part of a medical examination to check for pregnancy. Serum and urine hCG in early pregnancy is primarily hyperglycosylated with mean proportions of hyperglycosylated hCG in serum at the third, fourth, fifth, and sixth weeks of gestation of 90%, 54%, 42%, and 29% of total hCG respectively [Cole LA, 2003]. The predominant form of hCG in the second and third trimesters of pregnancy becomes regular hCG with a very minor portion (<2%) being hyperglycosylated hCG [Cole LA et al, 2007]. The hCG b-core fragment is the predominant form of hCG in urine from seven weeks of pregnancy until term[Cole LA et al, 2009]. In addition, the free b-subunit is also present in early pregnancy, becoming a minor component (<1%) of total hCG during the remainder of pregnancy.

Failed pregnancies: Generally, a rapid increase in hCG follows an intrauterine pregnancy. The hCG-doubling test is used as an indicator of pregnancy failure, miscarriage, or ectopic pregnancy in the period between four and seven weeks of gestation [Batzer FR et al, 2007]. The hCG-doubling test involves determining if the hCG serum level has doubled after two serum hCG measurements obtained 48 hours apart. It is used in individuals with fear of miscarriage, previous history of miscarriage, or infertility problems. After the hCG-doubling test confirmation, methotrexate is used to destroy and abort the ectopic pregnancy or a salpingectomy is performed.

Screening for Down syndrome: hCG is used in combination with a-fetoprotein and unconjugated estriol as part of a triple test to screen for the risk of Down-syndrome pregnancy, or those warranting the risk of amniocentesis in the second trimester of pregnancy. Improvement in the sensitivity for detecting Down-syndrome pregnancy was achieved with the addition of dimeric inhibin A as a fourth marker in a quadruple screen [Wald NJ et al, 1996]. The combination of ultrasound nuchal translucency with laboratory measurement of hCG and pregnancy-associated plasma protein (PAPP)-A became the standard to assess risk for Down-syndrome fetus. While a positive test with this combination of markers is the optimal prediction, it still only indicates approximately one in 20 chance of having a Down-syndrome fetus, therefore chorionic villous sampling or amniocentesis is warranted to give a definitive prediction.

Other malignancies:

Despite test manufacturers advising against the use of their tests for cancer cases, variants of hCG are used as markers of germ cell and other non-trophoblastic malignancies including testicular choriocarcinoma, testicular yolk-sac carcinoma, testicular embryonal carcinoma, and testicular teratoma. Free b-subunit in serum and the b-core fragment in urine could be measured as part of an annual physical examination to exclude cancer. It is common for macrophage enzymes in non-trophoblastic malignancies to degrade the molecules produced yielding multiple degradation products of free b-subunit. Thus, it is important for hCG assays to detect nicked molecules and hCG, and free b-subunit with cleaved C-terminal peptides. It is important that an appropriate total hCG test is being used for cancer management.

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