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Determination of human chorionic gonadotropin

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Determination of human chorionic gonadotropin (hCG) is used for diagnosis and monitoring of pregnancy, pregnancy related disorders, for trophoblastic and some nontrophoblastic tumors. In addition, hCG is determined for doping control in males. Assay of hCG is complicated by the occurrence of different molecular forms, which are detected to various degrees by different assays. The main form of hCG in circulation and in patients with trophoblastic tumors is intact heterodimeric hCG. The free β subunit (hCGβ) is a minor form in plasma in both conditions, but it may be the major form aggressive trophoblastic cancer. Therefore, assays measuring hCG and hCGβ together are mainly used for diagnosis of pregnancy and trophoblastic diseases. When excreted into urine, most of hCG (and hCGβ) is broken down to the core fragment of hCGβ (hCGβcf), which is the main immunoreactive form of hCG in urine during pregnancy. Specific determination of hCGβ is of value in screening for Down's syndrome and diagnosis of nontrophoblastic cancer. hCGbcf is of limited utility but it is important because it may disturb assay of hCG in pregnancy.

Introduction

Human chorionic gonadotropin (hCG) is a placental hormone necessary for the maintenance of pregnancy. Already in early pregnancy, trophoblasts secrete large amounts of hCG and determination of the concentrations in serum and urine is used to detect pregnancy and pregnancy related disorders.

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hCG and its subunits are also produced by virtually all trophoblastic tumors and most germ cell tumors of the gonads [1]. Determinations of hCG and its β subunit (hCGβ) are very important for monitoring of these diseases. In addition, hCGβ is also produced at low concentrations by many nontrophoblastic cancers, for which hCG is an independent prognostic marker [2]. hCG is also produced at low concentrations by the pituitary and can be measured in serum of healthy subjects by highly sensitive assays. In women, the serum concentrations increase after the menopause to levels similar to those in very early pregnancy. For diagnosis of pregnancy, hCG is often measured in urine with pregnancy tests [3]. Urine mostly contains an excess of a degradation products, i.e., the core fragment of hCGβ (hCGβcf) that can disturb the assay of hCG [4].

hCG structure

hCG consists of two noncovalently linked subunits, hCGβ and hCGα. The latter is common to all glycoprotein hormones (GPHs), luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). Hence it is also called GPHα. The β subunit of hCG and LH are highly homologous and LH and hCG activate the same LH/CG receptor. hCGβ contains 145 while LHβ contains 120 amino acids (aa). The C-terminal peptide comprising aa 121–145 is unique to hCG while the core regions of LH and hCG comprising aa 1–120 are highly similar, the homology between these being about 85%. Therefore, many antibodies to hCG also recognize LH and *vice versa*. About 30% of the weight of hCG consists of carbohydrates. hCGβ carries two N-linked glycans at Asn13 and Asn30 and four O-linked glycans linked to Ser121, Ser127, Ser132 and Ser138. The α subunit contains 92 amino acids and two N-linked carbohydrate chains on Asn52 and Asn78. The carbohydrate structure of hCG produced by various tissues may vary considerably [1]. hCG produced in early pregnancy and in cancer contains larger and more highly branched and more extensively sialylated glycans than that produced in mid and late pregnancy [5–7].

Forms of hCG in biological fluids

Different molecular forms of hCG occur in circulation and in urine, i.e., intact hCG, nicked hCG (hCGn), hCGβ and nicked hCGβ (hCGβn), the core fragment of hCGβ (hCGβcf) and hCGα [1]. Standards for these six variants have been prepared and initially established as International Reference Reagents (IRR) and the hCG preparation as the 5th International Standard (IS) by the WHO (Table 1). These preparations have been calibrated in substance concentration, i.e., mol but conversion factors to IU have been established by immunoassay [8,9]. When determined by immunoassays, the expression of hCG concentrations in units based on bioactivity is problematic. Immunoassays do not measure bioactivity but the concentrations of epitopes recognized by the antibodies used in the immunoassay. Thus, partially degraded forms of hCG, e.g., hCGn, hCGβ and hCGβcf, which lack bioactivity are recognized by many immunoassays [10–12]. Thus, when new and more pure international standards (IS) are introduced, the ratio of bioactivity to mass increases. In the 3rd (and identical 4th IS) the ratio of bioactivity to mass is 9200 IU/mg while in the most recent International Research Preparation (IRP 99/688) it is 10,300–15,400 [13]. The ratio between immunoreactivity and bioactivity is also changing with new hCG standards and the result is dependent on the immunoassay used. The 1st IRP has recently been adopted as the 5th IS for hCG. A conversion factor of 12,240 IU/mg has been determined by immunoassay.

Table 1
Major variants of hCG their abbreviations and MWs.

	Abbreviation	MW	pmol/IU
Human chorionic gonadotropin	hCG	37,500	2.9
β subunit of hCG	hCGβ	23,000	42.5
Core fragment of hCGβ	hCGβcf	13,000	
hCGα	hCGα	14,000	71.4