The Clinical Value of Serum Human Chorionic Gonadotropin

Agbecha, A
Department of Chemical Pathology,
Federal Medical Centre, Makurdi,
Benue State, Nigeria.
Corresponding author's Email: agbecha@gmail.com

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ABSTRACT
Human chorionic gonadotropin (hCG) is produced primarily by differentiated syncytiotrophoblasts, and represents a key embryonic signal that is essential for the maintenance of pregnancy. The hCG molecule is produced by a variety of organs, exists in various forms, and exerts vital biological functions. The hCG molecule displays specialized roles in promoting angiogenesis in the uterine endothelium, maintaining myometrial quiescence, as well as fostering immunomodulation at the maternal-fetal interface. The measurement of serum hCG levels provide important information in a variety of clinical situations ranging from diagnosis and monitoring of pregnancy, prenatal screening and pregnancy-related disorders to cancer surveillance. This review presents a biochemistry of hCG and its various clinical value.

Keywords: *human chorionic gonadotropin (hCG), prenatal screening, germ cell tumor, trophoblasts, pregnancy, clinical value*

INTRODUCTION

Human chorionic gonadotropin (hCG) is produced primarily by differentiated syncytiotrophoblasts, and represents a key embryonic signal that is essential for the maintenance of pregnancy. hCG is the major pregnancy glycoprotein hormone, whose maternal concentration and glycan structure change all along pregnancy [1]. It is detectable in maternal blood two days after implantation and behaves like an agonist of LH stimulating progesterone secretion by the corpus luteum. hCG has also a role in quiescence of the myometrium and local immune tolerance. Depending on its source of production, glycoforms of hCG display different biological activities and functions that may be considered as a placental hormone, an autocrine, a pituitary hormone and a major cancer promoter. Determination of hCG in serum and urine is used for the diagnosis and monitoring of pregnancy, prenatal screening, pregnancy related disorders, for trophoblastic and some nontrophoblastic tumors [1].

BIOCHEMISTRY AND BIOLOGICAL FUNCTIONS OF hCG

In early pregnancy, hCG is produced primarily by differentiated syncytiotrophoblasts, and represents a key embryonic signal essential for the maintenance of pregnancy. Serum

concentrations increase progressively in early pregnancy then peaks at 7 – 9 weeks of gestation and decreases until approximately 24 weeks then plateau (figure 1). During the initial six weeks of pregnancy, hCG promotes secretion of progesterone, estradiol, and estrone via transformation of the post-ovulatory ovary into the gravid corpus luteum [2]. Furthermore, hCG binds to its receptor to perform specialized roles in promoting angiogenesis in the uterine endothelium, maintaining myometrial quiescence, and enhancing immunomodulation at the maternal-fetal interface [2]. Immunomodulation at maternal-fetal interface is achieved by alteration of activity of dendritic cells, the reduction of T-cell activation and cytokine production, promotion of T regulatory (Treg) cell recruitment, and an increase in proliferation of uterine natural killer (NK) cells [2]. Metabolism of hCG by the placenta, liver, blood, and kidney determines its steady-state levels. 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 [3].

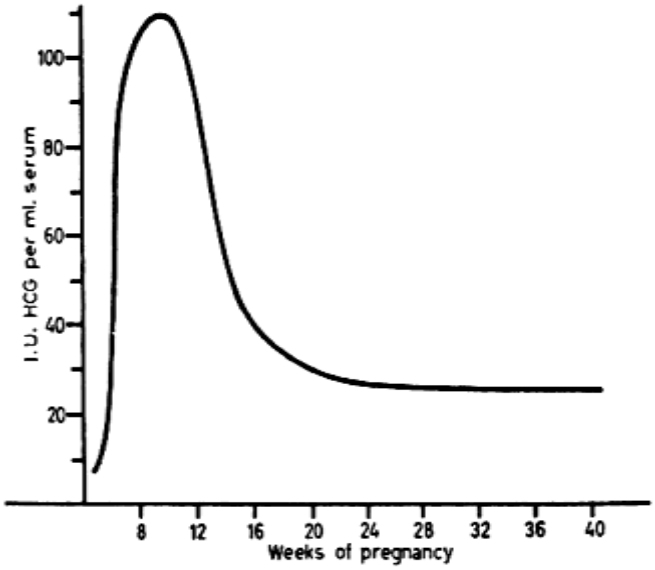
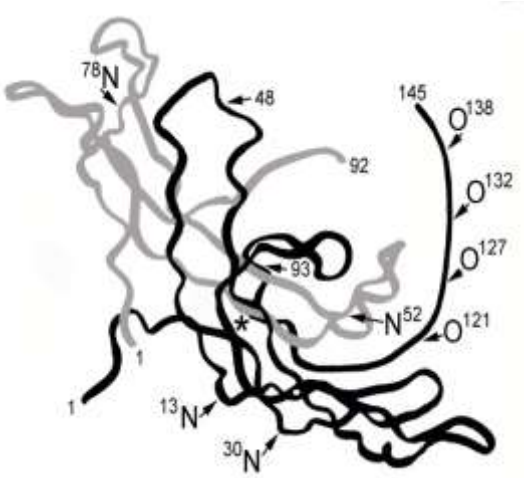


Figure 1: Serum concentration of hCG during pregnancy

Human chorionic gonadotropin is a heterodimeric glycoprotein of molecular weight 37,180, comprised of alpha (α) and beta (β) subunits that are non-covalently linked by carbohydrate side chains (figure 2). The α-subunit of hCG comprises 92 amino acids and 2 N-linked (Asn-linked) oligosaccharides encoded by a single gene (CGA) [3]. The ß-subunit comprises 145 amino acids, 2 N-linked (Asn-linked) and 4 O-linked (Ser-linked) oligosaccharides encoded by a cluster of genes (CGB) [3]. It shares some homology with luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and follicle-stimulating hormone (FSH) [2]. The α-subunit is identical to the pituitary gonadotropins (LH, FSH, TSH), while the



O and N- O-linked and N-linked oligosaccharides ,
*- the site of the cystine knot, common to TGFß.
Grey is α- and black is ß-subunit.

Figure 2: The structure of hCG

β-subunits are distinct in each of the homologous hormones which confer receptor and biological specificity [2]. However, the β-subunit is 80–85% similar to LH with a resultant consequence of several hCG antibodies recogninzing LH, and vice versa during immunoassay [4]. As a result of its structural similarity to LH, hCG binds to luteinizing hormone/chorionic gonadotropin receptor (LH/CGR) during the first 3–4 weeks of pregnancy, stimulating corpus luteal cells until the steroidogenic activity of the placenta produces sufficient progesterone to maintain pregnancy [5]. Other than LHCGR, hCG via autocrine and paracrine pathway induces transforming growth factor-β receptor (TGFβR) mediated signaling. Human chorionic gonadotropin also induces a weak hyperthyroid state in early pregnancy due to its similarity to TSH and as a result binds to thyroid stimulating hormone receptors on thyroidal cells. The biological functions of hCG during pregnancy are summarized in table 1.

Table 1: The biological functions of hCG during pregnancy

hCG variant	Function

Hcg	1. Promotion of corpus luteal progesterone production 2. Angiogenesis of uterine vasculature 3. Cytotrophoblast differentiation 4. Immuno-suppression and blockage of phagocytosis of invading trophoblast cells 5. Growth of uterus in line with fetal growth 6. Quiescence of uterine muscle contraction 7. Promotion of growth of fetal organs 8. Umbilical cord growth and development 9. Blastocysts signals uterine decidua prior to invasion regarding pending implantation 10. hCG in sperm and receptors found in fallopian tubes suggesting pre-pregnancy communication 11. hCG receptors in hippocampus and brain stem, may cause nausea and vomiting in pregnancy
hCG-H	1. Stimulates implantation by invasion of cytotrophoblast cells as occurs at implantation of pregnancy 2. Stimulates growth of placenta by promoting growth of cytotrophoblast cells
hCG and hCG-H together	Drives hemochorial placentation

hCG variants

The secretion, half-life, and functions of hCG all depend on its glycosylated state. Specifically, carbohydrate side chains play vital roles in its receptor binding affinity, function, and clearance from the circulation. Post-translational modifications of hCG result in three dimeric isoforms: "regular or intact (hCG), sulfated hCG (hCG-S), and hyperglycosylated hCG (hCG-H). Hyperglycosylated free beta (βhCG-H) and hCG free beta (βhCG) are degradation products of hCG-H and intact hCG respectively. hCG, sulfated hCG, hyperglycosylated hCG, hCG free beta and hyperglycosylated free beta. hCG variants are the most sialylated glycoproteins with up to 15 sialic acid residues per molecule. hCG variants are the most glycosylated of glycoproteins, hCG containing 30% sugar by molecular weight, hCG-H containing 39% sugar and βhCG-H containing 42% sugar by molecular weight. With these extreme molecular weights, hCG is the longest lasting circulating molecule in human blood with a circulating half life of 36 hours.

The hCG variants are produced by placental syncytiotrophoblast cells and pituitary gonadotrope cells (group 1), and by placental cytotrophoblast cells as well as human malignancies (group 2). These variants have independent functions, such that hCG may be considered as a placental hormone, an autocrine, a pituitary hormone and a major cancer promoter. Group 1 hCG variants are both hormones that act on the hCG/LH receptor and are central to human menstrual cycle and human pregnancy. Group 2 variants are autocrines that act by antagonizing a TGFβR and are critical to all advanced malignancies. The pituitary is the

predominant of source of hCG-S secretion and controls steroidogenesis during the menstrual cycle by inducing theca cell androstenedione production, corpus luteal progesterone production and by contributing to the process of ovulation. During pregnancy, hCG-H and hCG lead the implantation of placenta tissue into the uterus and the formation of villous trophoblast tissue. Syncytiotrophoblast derived hCG induces corpus luteal cells to produce progesterone, whereas cytotrophoblast-derived hCG-H act as an autocrine and paracrine factor by activating the transforming growth factor-β receptor (TGFβR) mediated signaling [6]. The hCG-H produced early in pregnancy and by various cancers contains more structurally-complex carbohydrates than hCG-H produced in mid and late pregnancy [4].

hCG-H and βhCG pathway is the center-point of all advanced human cancer biology, driving cancer growth, cancer invasion and cancer malignancy. There are seemingly 2 kinds of cancer as relates to hCG variants. Type 1 is a cancer of hCG-H producing cells; choriocarcinoma, gestational trophoblastic neoplasm, ovarian and testicular germ cell cancers. Type 1 cancer produces hCG-H from the start of malignancy that induces a cell transformation mechanism via antagonism of TGFβR [4]. Type 1 cancer is modulated completely by hCG-H by enhancing its growth, metastases and grade. Type 2 cancers represented by all other human malignancies; lung cancer, breast cancer, leukemia, lymphoma, start out as transformed cell driven by an hCG-independent process. As the cancer progresses and becomes advanced it is able to express the hCG ß-subunit gene and make free βhCG . The βhCG driven TGFβR antagonism mechanism takes over control of the cancer. In advanced cancer cells, highly expressed βhCG and hCG-H variants acts in an autocrine fashion to antagonize TGFβR, promoting cell growth and blocking cell apoptosis. As a result of the antagonism, collagenases and metalloproteinases are produced by cells favouring cancer metastasis [5, 6].

Degradation products of hCG molecule

Other molecular forms of hCG present in maternal serum and urine include dissociated or degraded molecules with no biological activity (figure 3). Key β subunit containing hCG isoforms in urine and blood include; non-nicked hCG, nicked hCG, free β subunit, non-nicked free β subunit, nicked free β subunit, and β-core fragment (only urine). Regular free α subunit and large free α subunits are the only α subunit containing hCG isoforms found in urine and blood. Serum free β -subunit (βhCG and βhCG-H) and urine β-core fragment are used as tumor markers for detection of malignancies [5]. hCG is measured by either the total hCG test, which measures all hCG variants including its β-subunit, or by an intact hCG assay, which measures dimeric molecules only. Most laboratory total hCG tests available in the market detect hCG, βhCG and other hCG degradation products and variants [5].

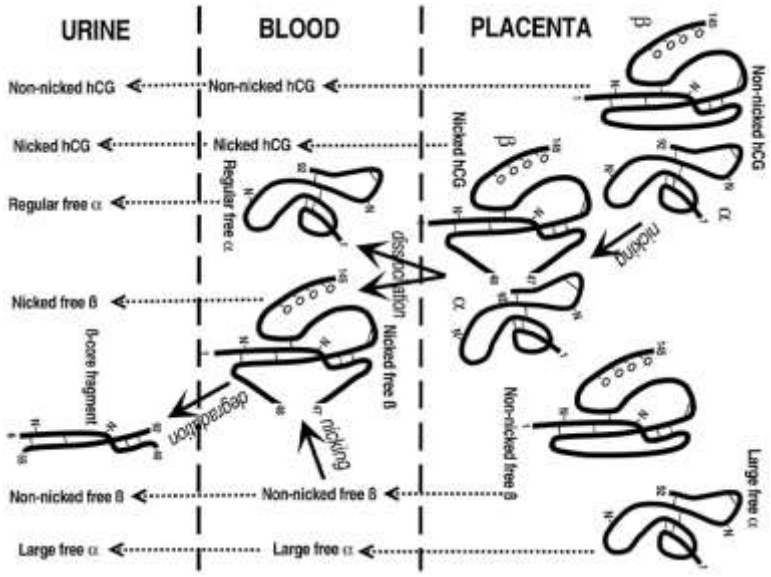


Figure 3: Molecular forms of hCG present in maternal serum and urine

THE VALUE OF hCG IN PREGNANCY VIABILITY TEST

A threatened abortion or a pregnancy of unknown location (ectopic pregnancy) can be accurately monitored by serial measurements of serum hCG levels compared to references in normal pregnancy (table 2). A viable intrauterine pregnancy may be accompanied by an hCG rise of >50% within 1.5-2 days interval (Case study 1). However, an increase in serum hCG levels of <35% is considered to be a safer definition of non-viability in a patient with a probable/likely ectopic pregnancy [1].

Table 2: Sample reference data of hCG in pregnancy

Weeks from conception	hCG (mIU/mL)
1	20 – 60
2	60 – 200
3	200 - 2,000
4	2,000 - 20,000
5-12	20,000 - 320,000
2nd trimester	20,000 - 60,000
3rd trimester	800- 30,000

Case Study 1: Monitoring pregnancy viability using hCG

- A 30 year old woman
- Pregnant by home urine test
- Presents with some vaginal bleeding at A & E
- Gestational age estimate = 4 weeks

Serial hCG (mIU/mL)
Day 1 3,000
Day 2 4,720
Day 3 7,590
Is this a viable pregnancy? YES!
hCG should rise by a minimum of 53% every 48 hours in early pregnancy

THE VALUE OF hCG IN PRENATAL SCREENING

Maternal serum human chorionic gonadotropin (MShCG) screening is a non-invasive biochemical test that provides information about the health status of the fetus [7]. MShCG could be measured along with other biochemical parameters as a triple or quadruple test including; unconjugated estriol [uE_3], inhibin A, pregnancy-associated plasma protein-A [PAPP-A] in the assessment of pregnancies at risk of aneuplodies. Nuchal translucency determined with the aid of ultrasonography may be combined together with the triple or quad test to improve the sensitivity and specificity of prenatal screening. Elevated levels of MShCG are observed in trisomy 21 (Down's syndrome) pregnancies while decreased levels are observed in trisomy 18 (Edward's syndrome) pregnancies [8].

Multiple of the Median hCG

In prenatal screening, since maternal parameters vary with gestation age (pregnancy stage), a separate reference interval has to be used for each week and sometimes day of pregnancy. To remove gestational age variation effects and to allow for some degree of standardization, biochemical and some biophysical (Ultrasound and Blood Pressure) parameters are expressed as Multiples of the normal Median. Multiples of the Median (MoM) is the ratio between the measured analyte concentration and the median concentration of the analyte in normal pregnancies at the same gestational age (equation 1). Thus if a MoM of 1.00 is the normal cut-off, a patients MoM 2.00 implies that the patients analyte concentration is 2 times higher than the reference median concentration and considered elevated, conversley a MoM 0.5 is interpreted as 0.5 times lower the median level and considered reduced. Thus, when the risk value for hCG in Down's syndrome screening is stated as being ' 2.5 times the median 'or ' 2.5 MoM 'this will be the same regardless of the week of pregnancy and it will be universal from center to center and assay to assay [8].

MShCG screening is usually determined between 16 and 18 weeks gestation; within this period, MShCG rapidly falls from 9 weeks to 24 weeks (figure 1 and table 3). Each laboratory is expected to develop reference data, with a median MShCG value (table 2) from unaffected pregnancies calculated for each week of gestation [7]. Maternal hCG levels of < 0.5, > 0.5 and < 2.0, and ≥ 2.0 MoM are usually categorised as low, normal and high, respectively [8].

Table 3: Medians for second-trimester maternal serum HCG

Gestational age (weeks)	hCG (IU/mL)
14	37.4
15	33.1
16	26.9
17	22.5
18	19.2
19	16.2
20	16.2

$$\text{MoM hCG} = \frac{\text{Patients hCG}}{\text{Median hCG}} \quad \text{..... equation 1}$$

In case study 2, patient 001 at 16 weeks of gestation for prenatal screening with a serum hCG level of 100 IU/ml will have a MoM hCG of 3.7 when calculated using a reference median hCG of 26.9 IU/ml. The MoM hCG of 3.7 implies that the patients serum hCG of 100 IU/ml is elevated by 3.7 times greater than the median reference of 26.9 IU/ml, and is predictive of Down's syndrome. Patient 002 is harboring a normal fetus. Patient 003 with an hCG value of 8.6 IU/ml would have MoM hCG of 0.32, implying that the patient's hCG of 8.6 IU/ml is reduced by 0.32 times less than the median reference of 26.9 IU/ml, which is predictive of Edward's syndrome. During prenatal screening maternal hCG levels of < 0.5, > 0.5 and < 2.0, and ≥ 2.0 MoM are categorized as low, normal and high, respectively. High and low values are considered abnormal and further subjected to invasive diagnostic procedures since their pregnancies are at high risk of fetal defect.

Case study 2; MoM hCG of patients at 16 weeks gestation

Patient's ID	Patient's hCG (IU/mL)	Median hCG (IU/mL) at 16 weeks gestation	Ratio	Patients MoM	Normal ratio (MoM)
001	100.0	26.9	100.0/26.9	3.7	1.00
002	26.8		26.8/26.9	1.00	
003	8.6		8.6/26.9	0.32	

THE VALUE OF hCG IN GESTATIONAL TROPHOBLASTIC DISEASE

Gestational trophoblastic disease (GTD) is a placental disease that arises from abnormal proliferation of trophoblastic cells in the placenta. When GTD persists or recurs it is often called gestational trophoblastic neoplasm (GTN). The spectrum of GTD includes: 1) Complete and partial hydatidiform molar pregnancies which is the most common form of GTD invasive mole (GTN). 2) Choriocarcinoma, placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT) which are all malignant degenerations of placental tissue. Very rarely

no antecedent pregnancy can be identified. Most, but not all GTD produce hCG, which is also produced in normal pregnancies and can be detected with a urine pregnancy test (UPT) [9].

Complete mole is the most frequent form (80% of GTD). Its chromosomal pattern is mostly 46XX and all chromosomes are paternal where often an 'empty' ovum is fertilized by a single sperm that duplicates, but sometimes two sperm cells fertilize an 'empty' ovum. No fetus is present in a complete molar pregnancy. In 15–20% of the complete hydatidiform moles, the trophoblastic tissue persists and causes persistent/invasive mole or choriocarcinoma [9].

In partial mole, two sperm cells fertilize a normal ovum resulting in a 69XYY, XXX or XXY chromosome pattern. Often a fetus or fetal tissue is present. Only 0.5–2% of partial moles develops into invasive moles [9].

Molar pregnancies are rare: approximately 1 in every 400–800 pregnancies is a complete or partial molar pregnancy. Choriocarcinoma and persistent trophoblastic neoplasm are even rarer with an incidence of approximately 1:50,000 pregnancies. Early signs and symptoms of GTD are mediated by elevated levels of hCG which include; severe hyperemesis, vaginal blood loss in first trimester of pregnancy, anemia, rapid growth of the uterus and early pre-eclampsia (before 20 weeks' gestation) [10]. Abdomen distention may be a sign of theca-lutein cysts or ascites, some women have respiratory failure or seizures (due to eclampsia or brain metastasis). In a number of women, small grape-like particles are born vaginally. Sometimes multiple ovarian cysts are seen and caused by hormonal stimulation of the ovaries by hCG. Five per cent of women present with hyperthyroidism since hCG has thyroid stimulating activity. In partial moles these symptoms are less pronounced as the hCG levels are lower compared to complete moles [10].

The value of hCG in prognosis, diagnosis, and monitoring treatment of GTD

Serial estimation of hCG in serum or urine is essential for diagnosis, monitoring of treatment and follow up. The hormonal level of hCG in blood and urine is increased by MoM >2.0 (more than two times the median hCG). hCG levels become undetectable within 3-6 weeks after normal delivery or non-molar miscarriage and 8-10 weeks after evacuation of molar pregnancy [10]. Persistence or rise after this period should therefore indicate residual or metastatic disease. During treatment, the level of hCG provides a guide for continuation or switch of chemotherapy agent/s. Follow up rising levels indicates relapse. However a false-negative urine pregnancy test (UPT) can be seen in molar pregnancies, therefore blood hCG levels are the gold standard in diagnosis and follow-up. It is recommended that an hCG assay that can detect all forms of hCG including beta-hCG, core hCG, C-terminal hCG, nicked-free beta, beta core, and preferably the hyperglycosylated forms, should be used [10].

Patients must be followed up with regular hCG after any diagnosis of molar pregnancy. In follow-up serum hCG should be checked every second week after evacuation of a molar pregnancy until it normalizes. Subsequently serum hCG is determined monthly for 1 year, when plateauing or rising hCG occur, GTN is diagnosed. Plateaued hCG is defined as four successive comparable measurements of hCG in 3 weeks. Rising hCG is defined as two consecutive increases in hCG concentration of ≥10%. In resource-poor settings the only way to detect recurrence is often UPT. The UPT is performed once monthly starting from the third to fourth

months until 1 year after evacuation of a molar pregnancy. The normal time for the hCG to normalize is 99 days in complete moles and 59 days in partial moles [11].

Chemotherapy is recommended when serum hCG of ≥ 20 IU/ml is observed 4 weeks after evacuation and when hCG is still present 6 months after evacuation. Very high levels of serum hCG in the order of >100 IU/ml is a high-risk factor for developing persistent mole (gestational trophoblastic neoplasia) in women diagnosed with GTD [11].

THE VALUE OF hCG IN PREGNANCY ASSOCIATED THYROID DYSFUNCTION

In normal pregnancy, hCG concentration induces a weak hyperthyroid state. The structural homology between hCG and TSH molecules as well as between their corresponding receptors provides the basis for the thyrotropic action of hCG [12]. Thyrotropic actions of hCG are mediated by its ability to stimulate TSH receptor on thyroid gland cells with the subsequent release of thyroid hormones. hCG concentrations physiologically peak in the first 8 to 11 weeks of pregnancy, decrease thereafter, and remain in plateau up to pregnancy term (figure 4). A negative correlation between hCG and TSH is characterized by increased hCG level corresponding with a low TSH level [13].

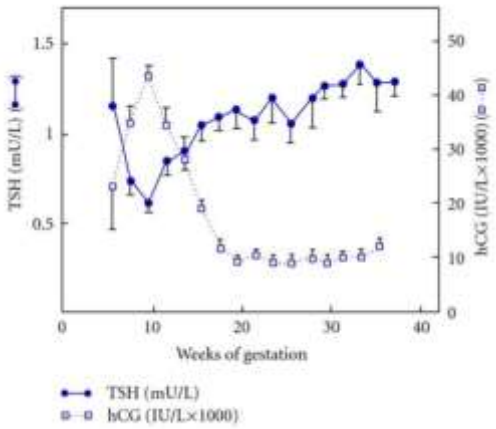


Figure 4: Relationship between serum hCG and TSH

Gestational transient thyrotoxicosis (GTT) refers to hyperthyroidism occurring in pregnant women without evidence of thyroid autoimmunity, which resolves spontaneously by the end of the 1st or early 2nd trimester of pregnancy. In most cases GTT is secondary to marked elevations of serum hCG in twin or multiple pregnancies, hyperplacentosis, hydatidiform mole, but it may also be due to circulating hCG isoforms with increased thyrotropic activity and/or prolonged half-life. In rare cases GTT could occur at normal levels of hCG, in this scenario hyperthyroidism is caused by increased sensitivity of TSH receptor to even minute quantities of hCG due to mutation of the TSH receptor (TSHR) gene [13].

hCG-induced hyperthyroidism has been reported in many patients with GTD. GTD-induced thyroid storm is a rare but potentially life-threatening complication of GTD. A high level of suspicion and early diagnosis in the emergency department is critical to obtain

appropriate treatment. This diagnosis should be considered in any female patient of childbearing age with signs and symptoms of thyrotoxicosis [14].

The beta subunit of hCG is structurally similar to TSH, allowing it to bind to the TSH receptor on thyroid follicular cells [15]. Of patients with hydatidiform mole, 25–64% has been reported to have increased thyroid function, but only about 5% have clinical signs of hyperthyroidism [16]. It has been reported that β-hCG levels of greater than 200 IU/ml sustained for several weeks are required to induce clinical hyperthyroidism [16]. It is estimated that for every 10 lU/ml increase in serum hCG, TSH decreases by 0.1 mlU/ml and free T4 increases by 0.1 ng/dL[15]. The weak hyperthyroid state in normal pregnancy is heightened in molar pregnancy due to higher concentrations of β-hCG. Additionally, the molecular variants of the β-hCG present in molar pregnancy have increased thyrotropic activity. The degree of increased thyroid hormone varies based on the level of β-hCG, the variant of β-hCG involved, and the duration of the molar pregnancy [14].

Clinical presentations can range from subclinical hyperthyroidism to thyrotoxicosis and thyroid storm. Thyroid storm is highly lethal, with a mortality of 10–30%, associated with tachycardia, fever, agitation, and altered mental status [17]. Patients are often profoundly tachycardic (>140 bpm), and have limited response to calcium-channel blockers, beta blockers, and intravenous fluids. A high level of suspicion and early diagnosis is crucial to prevent the complications of thyroid storm such as stroke, dysrhythmia, heart failure, rhabdomyolysis, liver dysfunction, and death due to multi organ failure [18].

The emergent management of thyroid storm involves decreasing hormone synthesis and release, blocking the action of thyroid hormone, reversing systemic decompensation (multi organ dysfunction), and removing the precipitating event [17]. Once patients are hemodynamically stable, the mainstay of definitive treatment is evacuation of the mole. Alternatively, hysterectomy is sometimes performed in patients who do not desire further pregnancy. The evacuation of the mole results in rapid reduction in thyroid hormone levels [11]. Post-evacuation follow-up with serial quantitative β-hCG measurements is crucial to evaluate for persistent molar tissue or development of choriocarcinoma. These complications develop in about 15–20% of patients with complete mole and 1–5% of patients with partial mole.

THE VALUE OF hCG IN GERM CELL TUMORS

Germ cell tumors (GCTs) are tumors involving the primordial germ cells of the gonads. The testis and ovaries are the most common primary sites where GCTs occur; however, the prevalence of GCTs is different in each of these sites. While over 90% of testicular tumors are GCTs and the most common cancers of young adult men, only 30% of ovarian tumors are GCTs. About 95% of all malignant testicular tumors are of germ-cell origin; the remaining are lymphomas, Leydig or Sertoli cell tumors, and mesotheliomas. Primary extragonadal GCTs also occur in the mediastinum (chest region), cranium (pineal/suprasellar region) and retroperitoneum (abdominal region). Embryologic and histopathologic considerations suggest two different origins of extragonadal GCTs: metastases of germ cells from gonadal GCTs and primary GCTs originating from adhered primordial germ cells during migration in embryonic life to the primitive gonads [19]. The mediastinum is the second most common primary site affected by

GCTs, with GCTs accounting for 15% of anterior mediastinal tumors in adults and 24% in children. GCTs also occur in the CNS, such as the pineal gland, neurohypophysis, and sacrococcygeal region [20]. GCTs metastasize via lymphatic and vascular routes. Extracranial GCT commonly metastasizes to the regional lymph nodes, and lungs (pulmonary visceral metastasis). Non-pulmonary visceral metastatic (NPVM) GCT sites include; liver, brain, bone, kidney, skin, gastrointestinal tract. Mortality is high in untreated GCT patients.

Varying pathologic subtypes of GCTs has been classified regardless of location but based on tumor marker and histological findings (table 3). GCTs are classified into two categories: Seminomas and non-seminomatous GCTs. Seminomas are sub-typed into germinomas and dysgeminomas, while non-seminomatous GCTs (NSGCTs) comprise of embryonal carcinomas, endodermal sinus yolk sac tumors, choriocarcinoma and teratomas.

Table 3 Classification of GCTs with serum AFP, hCG, and LDH

Germ Cell Tumor	Incidence	Tumor marker
Seminoma		
Seminoma/Dysgerminoma/Germinoma	30%–60% of GCTs	hCG may be elevated, LDH elevated, AFP normal
Non-seminomatous/non-germinomatous GCTs		
Embryonal carcinoma	3%–4% of GCTs	hCG and AFP elevated, LDH may be elevated
Yolk sac tumor (endodermal sinus tumor)		100% secrete AFP, LDH may be elevated, hCG normal
Choriocarcinoma	1% of GCTs	100% secrete hCG, AFP and LDH normal
Teratoma	5%–10% of GCTs	Normal hCG and AFP in pure teratomas, immature teratomas may secrete AFP and LDH
Mixed tumors		
Subtypes existing in a tumor	60% of GCTs	Depends on tumor cells present

Germ cell tumors may occur as mixed or pure tumors. There is a high prevalence of mixed tumors, such that a GCT at a given site in a patient may consist of different histologic subtypes of tumors. Pure embryonal carcinomas, yolk sac tumors, or choriocarcinomas are extremely rare, although seminomas often appear in a pure form. Approximately 60% of testicular tumors are mixed GCTs. The prevalence of mixed GCTs is lower in the ovary than in the testis [20].

hCG in the diagnosis of germ cell tumors

The diagnostic algorithm of GCTs includes a history and physical examination, cross-sectional imaging of the retroperitoneum, chest x-ray, and serum levels of AFP, hCG, and LDH [21]. Table 4 reviews some of the important characteristics of the common GCT markers.

Table 4 Characteristics of common GCT markers

Tumor marker	Half-life	Normal range	Tumor type
AFP	5–7 days	<40 µg/I	Embryonal, teratoma, yolk sac
hCG	24–36 hours	<5 IU/I	Seminoma, choriocarcinoma, embryonal,
LDH	Varies	1.5-3.2 ukat/I	Any tumor type

Determination of baseline serum hCG, AFP, and LDH levels before therapy is mandatory in all patients. The marker concentration in serum is dependent on histological type and tumor load (ie, stage). hCG is a marker of first choice for gonadal and extragonadal choriocarcinoma. hCG shows 100 % sensitivity for choriocarcinoma irrespective of their site. With regard to NSGCTs, hCG can be markedly elevated (in order of >10000 IU/L) with pure choriocarcinomas but is often only moderately elevated in conjunction with embryonal cell carcinoma and mixed GCTs. Elevations of hCG can also be seen in approximately 10%–20% of patients with stage I seminoma and up to 30%–50% of disseminated seminoma secondary to the presence of syncytiotrophoblastic elements within the tumor, but at serum levels rarely above 0.5 IU/ml [22]. The diagnosis of GCT cannot be made based on elevated tumor markers alone, as these markers lack sufficient sensitivity and specificity [22]. Diagnosis instead relies on the histopathologic examination of tissue specimen. There are however, unique clinical scenarios in which patients present with markedly elevated tumor marker levels and symptomatic metastases (eg, shortness of breath due to extensive lung metastases or severe retroperitoneal pain or edema), and biopsy for tissue diagnosis may not be advisable. In these situations, chemotherapy becomes the first line of therapy since these patients are categorized in the poor prognostic group based on tumor markers alone. Tumor markers may be the only clue to the tissue of origin if a biopsy of a metastatic site reveals only poorly differentiated carcinoma. These markers are also useful to indicate discrepancies in the pathologic diagnosis of GCTs [22]. For screening purposes, a combination of serum hCG and AFP levels revealed a prevalence rate of 60% in untreated stage-I, 70% in untreated stage-II and 90% in untreated stage-III non-seminomatous testicular carcinoma [23].

hCG in prognosis, risk stratification, and selection of GCT therapy

Other than differential diagnosis, serum hCG together with AFP are valuable markers of NSGCTs and have contributed significantly in terms of prognosis, staging, and making treatment decisions in enhancing the cure rate of these cancers [24]. Prognosis of GCTs relates not only to anatomic extent of spread, as is true for most cancers, but also relate to the primary site (extragonadal or gonadal) as well as the extent of production of the tumor markers; hCG, AFP,

and LDH, which may reflect the underlying biologic aggressiveness.

The degree of hCG, AFP, LDH elevation is directly proportional to tumor burden, these tumor markers thus serve as poor prognostic indicators associated with adverse outcome. Stage and prognostic stratifications are adopted in selecting appropriate therapy for patients in whom the diagnosis of cancer has been established. The extent of marker elevation in patients with NSGCTs has been incorporated into the American Cancer Society staging criteria (table 5) which categorizes patients into good, intermediate, and poor risk groups [25]. Also, the International Germ Cell Cancer Collaborative Group classify NSGCT into good, intermediate and poor prognostic groups using tumor markers, site of primary tumor (mediastinal or not), presence or absence of NPVM, and classify seminoma using NPVM alone (table 6) [26].

Table 5 American Joint Committee on Cancer Stage parameters

Stage	AFP (ug/I)	hCG (IU/I)	LDH
S0	normal	normal	normal
S1	<1000	<5000	<1.5 x ULN
S2	1000 – 10,000	5000– 50,000	1.5 – 10 x ULN
S3	>10,000	>50,000	>10 x ULN

Table 6 Classification of advanced (metastatic) GCTs into various risk groups using tumor markers

GOOD PROGNOSIS	
Non-seminoma (NS)	**Seminoma**
Primary testicular/retroperitoneal NS and Absence of non-pulmonary visceral metastases and Good markers - all of: AFP <1000 ng/ml and hCG<5000 iu/l (1000 ng/ml) and LDH <1.5 x ULN (upper limit of normal)	Any primary site and Absence of non-pulmonary visceral metastases and Normal AFP, any hCG level, any LDH level.
INTERMEDIATE PROGNOSIS	
Non-seminoma	**Seminoma**

Primary testicular/retroperitoneal NS and Absence of non-pulmonary visceral metastases and Intermediate markers-any of: AFP 1000 ng/ml to10,000 ng/ml or hCG 5000 iu/l to 50,000 iu/l or LDH 1.5 x ULN to 10 x ULN	Any primary site and Presence of non-pulmonary visceral metastases and Normal AFP, any hCG level, any LDH level.
POOR PROGNOSIS	
Non-seminoma	**Seminoma**
Primary mediastinal NS or Presence of non-pulmonary visceral metastases or Poor markers- any of: AFP >10,000 ng/ml or hCG>50,000 iu/l or LDH >10 x ULN	No patients classified as poor prognosis

The selection of treatment is based on patients risk level (low, moderate, high) which depends on tumor type, tumor stage and prognostic group. For example stage I seminomas and non-seminomas may be treated by surgery: orchiectomy (testis), oophorectomy (ovary). Surgery in combination with radiotherapy leads to a higher cure rate than surgery alone. Without radiotherapy some patients may relapse, but mostly cured by second-line therapy. Therefore, surveillance using tumor markers at an increased frequency to detect relapse is recommended.

The value of hCG in monitoring GCT therapy and follow-up surveillance

In all therapies, surveillance using tumor markers (hCG, AFP or LDH) at an increased frequency to detect relapse is recommended. If tumor markers in serum are elevated before therapy, their levels should be monitored weekly until concentrations are within the reference interval. Marker levels exceeding the upper reference limit after therapy suggest residual disease. The rate of marker decline reflects the response to therapy; a slow rate of marker decline may imply a need for more aggressive therapy [24]. Persistent marker elevation after chemotherapy indicates residual disease and the need for further therapy. In the absence of residual disease after surgery, the half-life of hCG is approximately 1.5 days and that of AFP is 5 days. During chemotherapy, half-lives >3.5 days for hCG or >7 days for AFP predict recurrence and adverse prognosis [24]. Half life of an analyte in the blood is defined as the amount of time (days) it takes for the initial concentration (level) of the analyte to decrease by half. In the evaluation of

hCG levels during monitoring of response to therapy, the elevated hCG is expected to drop by half in two days after surgical removal of the primary tumor. Knowledge of the half-life of these tumor markers is vital in timing sample collection during monitoring of therapy. It takes at least four days for hCG to reduce after commencement of chemotherapy [24].

During surveillance, serial monitoring with hCG, AFP, and LDH is recommended even when these are not raised prior to therapy as marker expression can change during therapy. Frequency of measurement depends on the stage and pathology of disease; patients with low-risk disease treated with surgery alone are monitored most frequently eg, every 1-2 weeks during the first 6 months in order to detect a relapse before tumor grows to a size associated with adverse prognosis, as estimated by serum concentrations of hCG >1,000 U/L and of AFP >500 kU/L [27]. In all patients, monitoring is continued for 5 years [28]. Because baseline levels are individual, increases are more important than absolute concentrations. A single increasing value must be confirmed with a second sample and the possibility of transient elevation due to non-specific interference (eg, iatrogenic hypogonadism) should be actively considered [24].

hCG in non-germ cell Tumor

Somatic cancers of various types occasionally develop from a teratoma and are classified as non-germ cell malignancies. Serum hCG levels are rarely elevated in non-germ cell tumors such as lung, breast, pancreas and bladder cancers [29].

hCG AS A BIOMARKER OF PREECLAMPSIA

Pregnancies complicated by preeclampsia display an over-abundance of non-invasive syncytiotrophoblasts accompanied by inadequate cytotrophoblast invasion. Preeclampsia is frequently accompanied by low hCG-H serum levels during the first trimester of pregnancy (8–13 weeks) [30]. Higher hCG levels were observed in serum from preeclamptic pregnancies at term compared with serum derived from normal pregnancies [31].

THE USE OF hCG IN ASSISTED REPRODUCTIVE TECHNOLOGY

In ART, hCG is used as a surrogate for LH. The mid-cycle LH surge is essential to achieve normal oocyte maturation and ovulation. Partially purified urinary hCG or recombinant hCG (r-hCG) preparations are administered into women to achieve final oocyte maturation and ovulation during controlled ovarian hyper-stimulation, and to facilitate correct timing of oocyte retrieval ART [32]. In assisted reproduction, hCG use may result in different responses that are more advantageous than LH responses with respect to mature oocyte collection, embryo quality, implantation and pregnancy rate. Administration of r-hCG is associated with significantly improved patient tolerance compared with urinary hCG administration [33].

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