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MINIREVIEW

Non trophoblastic source of human chorionic gonadotropin – problem in diagnostic accuracy

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Abstract

Human chorionic gonadotropin (hCG) is a polypeptide hormone studied as far as 1912, but researchers has no complete knowledge concerning its biological function. Since 1970' it is known that hCG can be found not only in the urine and serum of pregnant, but in choriocarcinoma and testicular cancer patients. Up-to-date one can distinguish four subtypes of hCG differing in secondary carbohydrate chains configuration as well as it regular and glycosylated forms, but non trophoblastic sources of this hormone, such as pituitary are still not widely known. The article gives an overlook on hCG studies in order to help clinicians in taking wise, evidence based decisions in asymptomatic patients with elevated hCG.

INTRODUCTION

Human chorionic gonadotropin (hCG) is a hormone, which was described and named before its chemical structure had been specified. Its existence was evidenced by the results of several experimental studies. The first studies were conducted by Archer in 1912. The experiments were based on stimulating the reproductive organs of guinea pigs with an aqueous solution obtained from human placenta. A year later, Fellner showed that similar extracts administered to sexually immature rabbit females, induce ovulation in their organisms. In subsequent experiments, which were published by Hirose in 1919, it was demonstrated that continuation of the regular injection of placental extract not only causes the release of the oocyte, but also induces the formation of the normal *corpus luteum*.

DISCUSSION

The above-mentioned studies have demonstrated the existence of at least one hormone, which has a key role in the biology of reproduction. Its chemical structure was described much later, barely in the 70's during the research on cancer diagnosis in the National Cancer Institute, constituting a part of the National Institute of Health in Bethesda (USA). In subsequent years, it was observed that hCG is detected not only in the urine of pregnant women, but also in patients with choriocarcinoma (Vaitukaitis *et al.* 1972).

Ross and Vaitukaitis attempted to determine the concentration of hCG with high accuracy in the tested serum. Until then, HCG and lutropin (LH), due to the similarity of the chemical structure, were determined together and referred to as

the luteinizing hormone. After three years of experiments, they managed to develop a suitable assay based on the radioisotopic labeling of antibodies derived from rabbit immunized with human β-hCG (Rayford *et al.* 1972). This experiment was a milestone not only in gynecology, but also in general immunology. Subsequent studies have contributed much to our knowledge of the biological function of chorionic gonadotropin and its subunits. Their exact molecular structure and spatial arrangement of individual molecules were described barely in the mid 90's by Lapthorne *et al.* who conducted research on the diagnosis of cancer diseases (Lapthorne *et al.* 1994).

HCG is a glycopeptide hormone composed of two glycosylated polypeptide subunits α and β connected by disulfide bonds. All glycopeptide hormones, FSH, LH, TSH and hCG have identical α-chain composed of 92 amino acids. Their beta chains differ in amino acid and carbohydrates composition, which determines their specific biological activity. With the use of crystallography, one can distinguish 4 subtypes of hCG, which share common β core. These differences arise from the different configuration of molecular bonds involving carbon atom between the secondary carbohydrate chains (Choi & Smitz 2013).

Unlike most bio-molecules, in the case of β-hCG up to 25–41% of mass is represented by secondary sugar chains (for glycosylated hCG, this ratio is up to 35–41%). Glycosylated hCG is a particularly sensitive marker of pregnancy, which allows to confirm fertilization even on 4–7th day. Its production begins with cytotrophoblast invasion. In the third week, 87% of hCG produced is present in a glycosylated form, during the next week its contribution reduces to 51%, and to 43% in the subsequent week (Cole *et al.* 2006; 2010).

Genetic studies have shown, that genes encoding the beta subunit of LH and hCG are located close to each other within the chromosome 19. Their DNA sequence is very similar, Hollenberg suggests, that genes which encode hCG arose form duplication of LH gene. Each of them has its own promoter region, differing in only 10% of amino acid sequence. In turn, the gene encoding the α subunit (CGA) was located on chromosome 6 (Policastro *et al.* 1986; Jameson & Hollenberg 1993; Hollenberg *et al.* 1994).

Today it is known, that the main role of chorionic gonadotropin is a synergistic support of secretion and action of progesterone. Its direct impact on embryo implantation, function of the placenta, transformation of uterine during pregnancy and development of the fetus, are still under investigation. An important function of hCG is to prevent apoptosis of trophoblast cells in early pregnancy (Cole 2009, Głodek *et al.* 2012).

Berndt, Herr and Shi showed, that after the implantation of an embryo, hCG plays a significant role in the development of spiral arteries and indirectly affects the development of uteroplacental circulation, essential for the normal development of pregnancy. hCG plays a critical role in the process of implantation and proper angiogenesis. Its low concentrations increase the risk of preeclampsia in the second and third trimester of pregnancy (Shi *et al.* 1994; Herr *et al.* 2007; Berndt *et al.* 2009). Moreover, it was shown, that during pregnancy, hCG exhibits immunomodulating effect – stimulates macrophages and activates B lymphocytes (Muzzio *et al.* 2014), increasing the resistance of mother and fetus against infection without activation of T cells capable of responding to antigens derived from father (Wan *et al.* 2007).

Beyond pregnancy, the role of β-hCG is unknown. At the end of the 70', based on the studies of *Ovalipes ocellatus* crabs, Maruo suggested that beyond pregnancy, its presence is a result of laboratory errors resulting from the use of trypsin. Today, we know that it is associated with familial occurrence of the excessive production of β-hCG in the pituitary of some women (Mauro *et al.* 1979). So far, in more than 40 studies, the excretion of chorionic gonadotropin along with an increase in the concentration of LH in the middle of the cycle, has been confirmed, however in the majority of women its concentration remains below the limit of sensitivity of standard tests. As evidenced by Hoerman, LH and hCG can be distinguished not only by adopting immunohistochemistry, but also thanks to the use of high performance liquid chromatography (HPLC). Despite the small difference in their mass, one can observe the difference in their retention time using a chromatographic column filled with silica gel (Hoerman *et al.* 1995).

Probably, hCG plays an anti-apoptotic function in the biology of certain cancers. As demonstrated in studies conducted by Yoshimoto, despite the reactivity with hCG receptors, a substance produced by tumor cells and during the physiological menstrual cycle, differs from a hormone excreted during pregnancy by lesser number of carbohydrate moieties (lower degree of glycosylation), with predominant excretion of the free β-subunit of hCG (Yoshimoto *et al.* 1979). Attention should also be paid to the studies by Chapekar, in which he showed that granulosa cells undergo growth after stimulation of LH, but do not respond to hCG administration (Chapekar 2001).

In the study conducted by Cole, one revealed the presence of β-hCG in 84% of menstruating women, its concentration increased proportionally to the concentration of LH. In the remaining 16% of women for whom no beta hCG was reported, no LH peak was also detected, and the concentration of LH remained at the low level throughout the whole cycle (Cole & Gutierez 2009). Based on these results, one concluded on the presence of the role of human chorionic gonadotropin in maintaining the peak of LH, its importance for the induction of ovulation, and the initiation of the production of progesterone by the *corpus luteum*. The importance of hCG for the proper function of the ovarian follicle also appears in the studies of other authors (Odell & Griffin 1987; Diaz *et al.* 1999).

Pituitary secretion of β-hCG has also been found in men after exogenous stimulation with gonadoliberin (GnRH). Pituitary hCG can easily be detected in female patients after removal of the appendages and during perimenopause (Cole *et al.* 2009). Based on experiments performed with 240 patients, Snyder suggests a limit of physiological concentrations of hCG as 14 IU/L in women at the age above 55. He also reported, that in the age group of 41–55 years, in women with FSH>20 IU/L, the results of β-hCG determination between 5 IU/L and 14 IU/L also remain irrelevant and should not be associated with any suspicion of pregnancy or cancer (Snyder *et al.* 2005). Chorionic gonadotropin is not a standard parameter evaluating the health status of a woman, therefore it is difficult to evaluate the prevalence of elevated, asymptomatic or unrelated with molar pregnancy concentrations of pituitary hCG.

In studies conducted by Cole, among healthy women aged 18–34, familial elevated concentration of pituitary hCG in 4 out of 405 was observed (Cole & Gutierrez 2009). In turn, Palmieri during his 23-years of observations, described 14 women with elevated concentration of β-hCG. In 10 of them, no pathology was observed (Palmieri *et al.* 2007).

Distinction between the pituitary gonadotropin and the gonadotropin derived from the trophoblast is extremely difficult – the only difference in the chemical structure is based on different proportion of sulfonated oligosaccharide chains, which is possible to confirm only by specialized laboratories (Birken *et al.* 1996).

With the increase in sensitivity of βhCG assays, it becomes problematic how to proceed in terms of elevated levels of this hormone in a woman who is not pregnant. According Gronowski, the concentration of the hormone above 5 U/L is present in 3.6% of women aged 41–55 years.

Carter suggested the use of βhCG determinations for the monitoring of vulvar cancer, however yet, it has not been accepted in widely used clinical practice (Carter *et al.* 1995). Typically, a positive human chorionic gonadotropin test result in a non-pregnant female patient implies the diagnosis of molar pregnancy. False positive result is associated with unnecessary chemotherapy or operation. The presence of chorionic gonadotropin does not always result from the production of this pituitary hormone. Immunoassays used for the determinations may exhibit cross-reactivity e.g. with heterophilic antibodies secreted in response to viral and parasitic infections.

The incidence of false positive results was determined at 1/10 000, and using a test with double binding site, at 1/1 000 000. Partially, they are the result of impossible to avoid contamination of laboratory equipment, and pituitary production of β-hCG as well. The reliability of the obtained result is difficult to assess in clinical setting, however generally, false positive results are characterized by a small increase in concentration, which is not confirmed by serial dilution method or any inconsistency of measurements in urine and serum (Tsai 2006).

A tip on how to confirm the source of pituitary chorionic gonadotropin secretion in the clinical settings, is described in the publication by Merhie. He described the case of a 31-year-old female patient with 46XY karyotype, primary amenorrhea and Fraser syndrome, for whom during the preparation for the embryo transfer (oocyte obtained from a donor), the concentration of β-hCG was reported at a level of 35 IU/L. During serial dilutions, no inconsistencies were found, the hormone was also present in the urine. However, no occurrence of the free β-subunit or hyperglycosylated gonadotropin were found. After stimulation with exogenous estradiol, during the preparation for the transfer, normalization of β-hCG concentration was achieved (Mehri & Pollack 2013).

This case suggests an association with the observed elevated levels of human chorionic gonadotropin in women with failure of gonadal function (Cole *et al.* 2009). Despite few publications on this topic, there is still no simple, clinically accepted test allowing to preclude pregnancy or cancer in 100%. As arise from reports presented by the University of Albuquerque which constitute the largest reference center in the United States, still a significant proportion of asymptomatic women is subjected to aggressive chemotherapy or hysterectomy. In the collected base involving 170 female patients with β-hCG concentration at the level of 6.9–900 IU/L persisting for more than three months, in only 13 (7.6%) malignant transformation was confirmed. Unfortunately, among 76 women with false-positive results and 17 with confirmed pituitary source of human chorionic gonadotropin, 9 and 47 women underwent hysterectomy and a full cycle of chemotherapy, respectively – of course without any influence on the hormone level. Considering the qualifications for the treatment, one cannot rely on the collected interview – 111 female patients had been previously diagnosed neither of any form of molar pregnancy nor in the family (Cole *et al.* 2006).

CONCLUSION

Data presented above show, how low awareness of clinicians in this issue is and how willingly they implement ineffective therapies with fear of overlooking choriocarcinoma.

However, a pattern of reference procedure, which allows to avoid unnecessary invasive procedures and at the same time capable of capturing the female patients from high risk group, has been described. It was proposed, that after the exclusion of intrauterine and ectopic pregnancy in female patients with chronic persistent β-hCG, it is advisable to conduct the assay in a different laboratory, necessarily utilizing systems from Abbott Architect, Roche Elecsys, or DPC Immulite, which are the least prone to false positive results (Tsai 2006). After

confirming, that indeed we deal with elevated level of human chorionic gonadotropin, and not with the test failure due to the above-mentioned cross-reactions, it should be established which type of gonadotropin predominates in a sample of a female patient.

Elevated concentrations of hyperglycosylated hCG indicates a malignant tumor, the presence of the free β-subunit of hCG in more than two thirds, speaks for site placental trophoblastic tumor, pancreas tumor or carcinoid (the attention should be paid to the characteristic clinical symptoms of these tumors). In case of the absence of significant concentrations of the above-mentioned hormones, the most probable diagnosis is benign trophoblastic disease, and in women during perimenopause or ovarian dysfunction, overproduction of a pituitary hormone. Unfortunately, these tests are not widely available for clinicians, therefore after excluding pregnancy and trophoblastic disease, female patients with elevated levels of hCG should be directed to centers having certified experience and capability to conduct highly specialized diagnostic tests.

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