# Human chorionic gonadotropin-beta in endometrium cancer tissue

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**Summary**

*Purpose:* Determination of the correlation between expression of human chorionic gonadotropin mRNA and serum free hCGb immunoreactivity in endometrial cancer tissue.

*Methods:* The study included 56 patients with endometrial carcinoma Stages IB-III. The expression of mRNA *hCGβ* was determinated by the RT PCR method in 18 cases of cancerous and precancerous tissues. The serum-free hCGβ immunoreactivity was analyzed by sequential immunometric assay in all patients.

*Results:* In 15 study specimens of endometrial carcinoma tissue mRNA of *hCGβ* was detected. Also in endometrial atypical hyperplasia, expression of *hCGβ* was found. Noncancerous tissue demonstrated lack of the *hCGβ* transcript. The serum immunoreactivity in the endometrial cancer group was detectable in 86% of cases. There were no significant differences between FIGO stages and grading.

*Conclusion:* The results of the present study confirmed the presence of active genes of *hCGβ* in endometrial cancer tissue, even in precancerous changes. The serum immunoreactivity of free hCGβ is a less common feature and is not linked with tumor stage or grade in endometrial cancer patients.

*Key words:* Human chorionic gonadotropin beta; mRNA of *hCGβ*; Endometrial carcinoma.

## Introduction

Human chorionic gonadotropin (hCG) is a sialoglycoprotein hormone, physiologically produced by syncytiotrophoblastic cells of the placenta, however the precise biological significance of hCG in pregnancy development is still unknown [1]. In gestational trophoblastic disease, hCG has been approved as an established tumor marker [2, 3]. In addition, serum and urine of patients with non-trophoblastic cancers, such as cancers of the ovary, cervix, gastrointestinal tract, bladder, lung reveal hCG and hCGβ immunoreactivity [4-9]. Acevedo and co-workers have shown that the presence of membrane-associated hCG is a common phenotypic characteristic of cancer [10, 11]. The serum immunoreactivity of hCG and hCGß has been observed in patients with gynecological malignancies [12]. Moreover, the urinary hCGβ core-fragment is present in patients with cancer of the ovary, vulva and vagina [13-15].

The aim of the present study was to determinate the correlating between the expression of *hCGβ* mRNA in endometrial cancer tissue and serum-free hCGβ immunoreactivity.

## Material and Methods

*Tissue samples*

*Endometrial cancer samples.* Surgical specimens of tissue were obtained from 18 patients with endometrial carcinoma (median age: 61, range 47-80) diagnosed as endometrial carcinoma by uterine curettage in different hospitals. In all women surgery was performed (total abdominal hysterectomy and bilateral salpingo-oophorectomy) at the Department of Gynecologic Oncology, Poznań University of Medical Sciences in 2002. Histological evidence, including tumor grading, was obtained and the staging was performed according to the International Federation of Gynecology and Obstetrics (FIGO). In three women the final histologic examination did not confirm the presence of cancer. In two cases endometrial hyperplasia without atypia was recognized, and in one case - with atypia. Histologically, all specimens showed the presence of the endometrioides adenocarcinoma (tumor grading: G1 - 13, G2 - 2; FIGO: IB - 10, IC - 2, IIA - 1, IIB - 2).

*Control group samples.* The control group consisted of tissue derived from the same patients' surgical specimens that were used to evaluate hCGβ in carcinoma tissue. That tissue lacked cancerous changes when it was evaluated under macroscopic examination. The control tissue of the salpinges (5 samples from endometrial cancer patients: FIGO - IB) and the uterine cervix (5 samples from endometrial cancer patients: FIGO - IB) were collected. The additional 15 samples of the endometrium without cancerous changes (histological: *proliferating endometrium* - 8, and secretory endometrium - 7) from women undergoing surgery due to myomas was availed as a control group.

*Positive control group samples.* Four placentas from term pregnancies served as a positive control. Placentas were obtained from the Department of Perinatology of Karol Marcinkowski University of Medical Sciences, Poznań, Poland.

*Specificity control.* To confirm the specificity of the used hCGβ primers tissue from the pituitary gland was applied as a control.

Tissue samples taken at the operation were frozen in liquid nitrogen. The qualification of each tissue sample was obtained by macroscopic pathology examination. No patients received chemotherapy or radiotherapy prior to surgery.

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*Serum samples*

*Endometrial cancer patients.* Preoperative serum samples obtained from 18 patients described above and additionally from 38 women with endometrial cancer (adenocarcinoma endometrioid type - 34, clear cell-type adenocarcinoma - 4; FIGO IB - 25, IC - 4, IIA - 2, IIB - 2, III - 5; G1 - 28, G2 - 6, G3 - 4 ) treated at the Department of Gynecologic Oncology of Karol Marcinkowski University of Medical Sciences in 2001.

*Control group.* To determine the serum-free hCGβ quantity the control group consisted of 55 women (mean age 42, range: 35-47) treated for benign disease. No patients had cancerous changes at any time.

*RNA extraction and cDNA synthesis*

Total RNA was isolated from the tissues with TRIZOL Reagent (GIBCO BRL, Grand Island, NY, USA), according to the manufacturer's protocol. RNA isolated from human placenta served as a positive control and endometrium, myometrium and cervices lacking cancerous changes served as negative controls. About 10 μg of RNA (DNase-treated) was employed individually for one reverse transcription reaction in a mixture containing: 50 pmoles of hCGβ sequence specific primer (5'-GAGAAGCCTTTATTGTG-3', nucleotides: 506-522, PubMed – AC: J00117) or 100 pmoles Oligo $(dT)_{10}$ primer, 5U/μl Expand reverse transcriptase, 1x Expand reverse transcriptase buffer, 1mM of dNTPs, 20U RNase inhibitor, 10 mM DDT. The reaction mixture was incubated at 42°C for 60 min, and the reaction was stopped by putting it on ice. All compounds used for cDNA synthesis were obtained from Roche Molecular Biochemicals, Mannheim, Germany. The primers were designed to be complementary to splice junctioning.

*PCR amplification*

A 210bp fragment of *hCGβ* was amplified from cDNA using the following primers: sense 5'-GCAGGGGACGCAC-CAAGGA-3' (nucleotides 8-27, according to cDNA sequence, PubMed - AC: J00117) and antisense 5'-CACGCGGGT CATG-GTGGG-3' (complimentary to nucleotides 200-217).

A specific fragment of *β-actin* gene was amplified from cDNA synthesized with universal oligo $(dT)_{10}$ primer using RNA isolated from endometrial carcinoma tissue and from genital tract tissue of the negative control group. The reaction was carried out in order to check the quality of RNA. A 604bp fragment of *β-actin* was amplified using the following primers: sense 5'-CATGTACGTTGCTATCCAGGC-3' (nucleotides 2057-2078, PubMed – AC: M10277) and antisense: 5'-CAGACAGCACTGCTGTGTTGGC-3' (nucleotides 2644-2661, PubMed - AC: M10277).

The amplification was performed in a reaction mixture containing: 1x Taq DNA polymerase buffer, 2.5mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM of each primer and one unit of Taq DNA polymerase, with a thermal profile as follows: 5 min at 95°C, 1 min at 95°C, 45 sec at a temperature specific for the primer set, 1 min at 72°C for 30 cycles. All compounds used for cDNA synthesis were obtained from Bioline, London, UK. The amplified products (half of the reaction mixture) were electrophoresed on 1% agarose gel (FMC BioProducts, Rockland, MA, USA).

*Assay*

Serum-free hCGβ was determined by a commercial sequential immunometric assay (Immulite Free Beta HCG, DPC Los Angeles, CA, USA). Results are expressed in ng/ml, where 1 ng/ml of free hCGβ is equivalent to 1 mIU of free hCGβ in terms of the World Health Organization's first international preparation of human chorionic gonadotropin beta subunit, number 75/551 (1st IRP 75/551). The analytical sensitivity of this kit is 0.02 ng/ml. The cross-reactivity of intact hCG in the assay for hCGβ was 0.089%, of hCGα - 0.15%, LHb - 0.4%, TSHβ - 0.002% and with FSHβ it was non detectable.

*Statistical analysis*

Statistical analysis was performed using Statistica version 5.0 kit (StatSoft Inc. USA). Associations between clinic-pathological parameters and free hCGβ levels required the Mann-Whitney U-test and Kruskal-Wallis test.

## Results

*Tissue expression of mRNA hCGβ*

*Endometrial cancer group.* A specific region of beta-subunits of human chorionic gonadotropin was amplified from total RNA through reverse transcription followed by polymerase chain reaction (RT-PCR). Primers specific for human *hCGβ* were used and 210 bp fragment of the *hCGβ* was detected in cases of positive control – human placenta (Figure 1; lines 1-2 and Figures 2, 3 and 4; lines 1 and 7, respectively).

In 15 out of 18 carcinoma tissues of the endometrium a specific fragment of *hCGβ* was present (Figure 2; lines 2-4). In one obtained tissue of atypical hyperplasia we also observed the characteristic line of 210 bp in agarose gel (Figure 3; line 2).

*Control group.* PCR with cDNA synthesized from total RNA isolated from human placentas, run as a positive control, showed the presence of *hCGβ* transcripts. The pituitary gland tissue showed no *hCGβ* expression. PCR with cDNA synthesized from total RNA, isolated from women's genital tract (tissue without cancerous changes when estimated by macroscopic examination) and pineal gland tissue, run as a negative control, did not show the presence of *hCGβ* (Figure 4; lines 5, 6 and 4, respectively). In these cases, the RT-PCR amplification of *β-actin* gene gave positive results. A 604 bp fragment of *β-actin* was detected in all study cases, showing the presence of intact RNA (Figure 4; lines 1-3).

The choice of the control group allowed the determination that the expression of *hCGβ* is the property of tumor cells. The tissue, derived from the same surgical specimens without cancerous changes when estimated by macroscopic examination, did not show any signs of *hCGβ* expression.

*Serum immunoreactivity of free hCGβ*

*Endometrial cancer patients.* In women suffering from endometrial carcinoma (n = 56) an elevated level of free hCGβ was observed in 48 (86%) cases. In 17 patients the estimated value was at the limit of the method of sensitivity (0.02 ng/ml). The mean level of free hCGb was 0.030 ng/ml (SD = 0l022, range between 0.00 to 0.10). The comparison between the grading (G1, G2,) when evaluated by Mann-Whitney-U and Kruskal-Wallis tests was not statistically significant *($p > 0.05$)*. The correla-

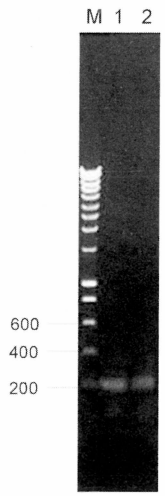


Fig. 1

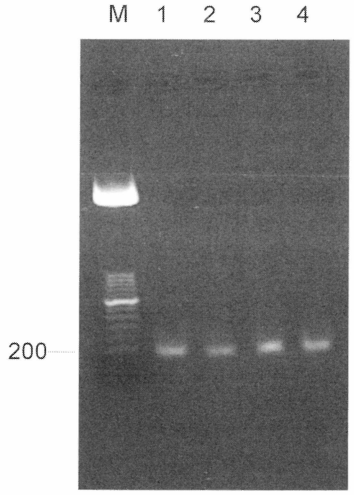


Fig. 2

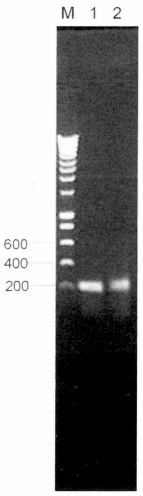


Fig. 3

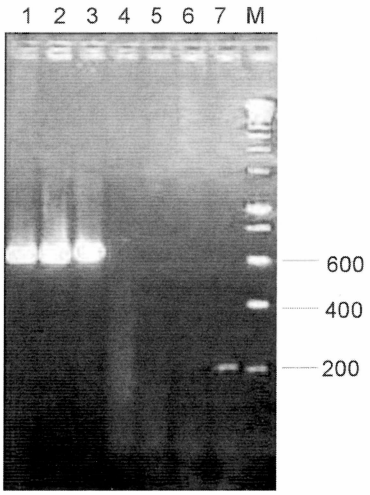


Fig. 4

Figure 1. — RT- PCR analysis of *hCGβ* in human placenta. Agarose gel resolution of products for *hCGβ* (210 bp) amplified from normal placenta - positive control - line 1; M – mass marker.
Figure 2. — RT- PCR analysis of *hCGβ* in endometrium carcinoma tissue. Specific fragment of *hCGβ* is shown in lines 2-4; in line 1 - positive control - placenta.
Figure 3. — Electrophoretic analysis of RT PCR products for tissue of atypical hyperplasia. A characteristic fragment of *hCGβ* was obtained - line 2.
Figure 4. — RT PCR analysis of noncancerous pineal gland tissue. No product was observed when the tissue of women's genital tracts lacked cancerous changes, lines 5, 6 and pineal gland - line 4, were analyzed. In these cases *β-actin* product (604 bp) is shown as a control of cDNA integrity - lines 1-3; line 7 - positive control - placenta.

tion between elevated free hCGb and FIGO stage of the endometrial cancer was also not statistically significant *($p > 0.05$).*

*Control group.*

In the control group (n = 55) the mean level of hCGβ was 0.014 (SD = 0.0185, range between 0.00 to 0.06). An elevated free hCGb level was observed in six cases.

Levels of hCGβ in cases of endometrial cancer in comparison with serum immunoreactivity of women from the control group showed statistically significant differences *($p = 0.0013$).*

## Discussion

The present study was undertaken to determinate the correlation between expression of *hCGβ* and serum immunoreactivity in endometrial cancers patients. The findings indicate that mRNA of *hCGβ* is present in endometrial carcinoma. This is, to our knowledge, the first report which directly confirms the production of hCGβ by endometrial carcinoma tissues. At the same time we showed that noncancerous tissue from the same women's genital tract demonstrated a lack of expression of the hormone. Thus, the present study shows that expression of hCGβ is a characteristic feature of tumor tissue. The results of serum immunoreactivity showed that endometrial carcinoma patients have elevated serum levels of free hCGβ. A mean level of the hormone - 0.052 ng/ml - was observed in 86% study cases. Correlations between hCGβ levels and FIGO stage and grading were not observed.

As previously demonstrated by Acevedo and Hartsock on a nude mice model, high expression of membrane-associated hCGβ correlated with primary malignant growth and metastatic phenotype of cancer [16]. The biological significance of this finding is still unclear. The discovery of the crystal structure of hCG demonstrated that dimeric hCG forms a cystine knot structure [17, 18]. That structure of hCG resembles the structure of known growth factors, namely TGFβ (transforming growth factor), NGF (nerve growth factor) and PGFβ (platelet derived growth factor), thus suggesting the role of hCG in tumor growth [19, 20].

The study by Rivera's group showed that applying the antisense RNA specific to *hCGβ* caused the loss of tumorigenic potential of human lung cancer cells cultured *in vitro* (ChaGo cells) [21].

It has been suggested that hCG may play a role in the immunologic tolerance of the host organism against cancer [22-24]. It has been shown that hCG expressed on tumor cell surfaces suppresses the action of T cells, allowing the proliferation and local invasiveness of cancer cells. Further investigations should allow us to determine what types of cancer tissue cells express *hCGβ* and to find out when the cells possess the ability to produce hCGβ in pre-neoplastic lesions. These findings seem to have some clinical importance. Recently, Gawronska *et al.* have shown that β subunits of hCG conjugated to the lytic peptide, hecate, destroyed ovarian cancer cells (OVCAR-3) [25]. The construction of antisense RNA or siRNA against hCGβ – which would block *hCGβ* re-expression – could lead to a new way to treat patients with gynecological cancers.

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