THE MODIFIED RADIOIMMUNOASSAY OF SERUM INHIBIN AND ITS VALUE IN MONITORING OVARIAN TUMOR

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ABSTRACT

Objectives and Methods: A modified radioimmunoassay (RIA) of serum inhibin (INH) was developed and applied to measure serum INH contents in 39 fertile and 16 postmenopausal women. Thirty-three cases of ovarian tumors, including granulosa cell tumors and other kinds of ovarian tumors, were monitored by serum INH RIA. Results: The mean value of serum INH contents in follicular, peri-ovulatory and mid-luteal phases of fertile women were 9.48 ± 7.10 pg/ml (2.04~18.53pg/ml), 19.04 ± 9.73 pg/ml (3.49~33.26 pg/ml) and 131.13 ± 110.81pg/ml (3.49~ 341.10 pg/ml), respectively. Serum INH concentration was negatively correlated with serum FSH concentration, (r_s= –0.483, P<0.01). Serum IHN contents were less than 3.6 pg/ml in normal postmenopausal women. The mean value of serum INH contents in ovarian granulosa cell tumor, thecoma, mucinous cystadenocarcinoma and malignant teratoma cases were significantly higher than that of other ovarian tumors, (P<0.01). Serum INH contents were elevated in ovarian granulosa cell tumor, thecoma, mucinous cystadenocaricinoma and endometrioid carcinoma cases with serum CA-125 values in normal range before operation, but serum INH contents decreased to normal range within one week after operation. And consecutive serum INH RIA could be a valuable tool in monitoring for therapeutic effect. Conclusion: Modified INH RIA was of convenient, time-saving and quantitative characteristics, especially with its high sensitivity (< 1 pg/ml). There was a regular change of serum INH concentrations during menstrual cycle. INH could inhibit the synthesis and secretion of follicle stimulating hormone (FSH). INH would become a valuable marker for ovarian tumor. INH RIA combined with the measurement of serum CA-125 would be helpful to the early diagnosis, treatment and follow-up for ovarian cancer.

Key words: Inhibin, Ovarian tumor, Granulosa cell tumor, Radioimmunoassay, CA-125

Inhibin (INH) is a kind of polypeptide glycoprotein hormone secreted from ovarian granulosa cells. It's chief physiological effect is to inhibit production and secretion of follicle stimulating hormone (FSH) by anterior pituitary gland. There have been some reports[1-3] which suggested that the determination of serum INH contents would be valuable for monitoring ovarian cancer, trophoblastic disease and Down's syndrome. To make a widespread application in clinic, a convenient, sensitive, and accurate method is needed for monitoring serum INH levels. In this study, we established the modified INH radioimmunoassay (RIA) and it was applied to assay serum INH concentrations during follicular phase, peri-ovulatory phase, mid-luteal phase in fertile women and menopausal women, respectively. We also evaluated its value by monitoring serum INH levels during different periods in 33 cases with various ovarian tumors.

MATERIALS AND METHODS

Clinical Data

Thirty-nine fertile women aged 23~41 (28.72 ± 6.59) years, with normal menstrual cycles were randomly chosen. No uterine or ovarian disease was diagnosed in the group. Serum FSH, luteinizing

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hormone (LH), estradiol (E_2) and INH concentrations were determined in follicular phage (7~9th day), peri-ovulatory phase (13rd~15th day) and mid-luteal phase (20~22nd day). Serum FSH levels were more than 30 μ/l in sixteen healthy menopausal women aged 45~73 years, averaged 56.27 ± 8.7 years, with one year or more menopausal period. The study group consisted of thirty-three cases with various ovarian tumors which included three cases of granulosa-cell tumor, two cases of thecoma, six cases of mucinous cystadenocarcinoma, six cases of serous cystadenocarcinoma, two cases of endometrioid carcinoma, four cases of immature teratoma, four cases of mature teratoma, two cases of Krukenberg's tumor. Sixteen were menopausal women and seventeen were fertile women. Serum samples were collected to determine INH and cancer antigen-125 concentrations one week before and after operation. And all the cases were monitored by these two markers in 2~23 months follow up; averaged 14.3 months.

Reagents and Confectings

Purified porcineTyr-INH (No. 7282) with 32kDa molecular weight is the product of American Peninsula Lab. It was diluted with phosphate buffer solution (PBS) in succession to different concentrations in 0.1, 1, 4, 16, 64, 256, 1024 fmol/100μl solution, pH 7.4. INH-Ab (No. p53), product of American Peninsula lab, working concentration 1: 10000, ending concentration 1: 50000. $Na^{125}I$ was provided by Radiochemical Center in U.K, ^{125}I-INH was labeled by chloramine T procedure. Radioactivity-counting in 100μl of ^{125}I-INH reached 9000cpm. The ratio of radioactivity was 692.4 μCi. Goat anti-rabbit gamma globulin (IgG) used as second antibody was dissolved by 40 ml of double distilled water.

INH RIA

A pool of 1 ml serum sample from peripheral blood was added into a tube which contained anticoagulants, 20 μl of 0.3 mol/L ethylene diamine tetraacetic acid, disodium salt (EDTA• 2Na) and 500 μ of aprotinin; Centrifuged immediately (4°C, 3000 rpm, 5 min) after immingled, the serum was collected and stored at -20°C~40°C. Setting double standard and sample tubes based upon balance saturation procedure, and 100 μl of each preparations of standard INH, samples, ^{125}I-INH, and INH-Ab were added into each tube; Gross reaction volume reached 500 μl. The contents were incubated overnight at 4°C, and 500 μl of second antibody (goat anti-rabbit gamma globulin) was added into each tube, then pooled completely and retained for 45 min at room temperature; then the contents were centrifuged for 10 minutes at 3000 rpm and the precipitate was counted in a gamma counter (Sigma Corp.), 60 seconds per tube. Data were inputted into computer and analyzed in four arguments regression, and analytic equation was obtained in $y=1699.22x^3+0.58x^2+56.55x-381.01$. Standard curve (omitted) was delineated with X-axis by concentrations of standard preparations and Y-axis by B/Bo. R=0.99, Bo=1810, ED_{75}=146.97 ED_{50}=1493.04, ED_{25}=15380.66, in fmol/100μl unit, or 1 foml/100μl=3.6 pg/ml convertioned through international unification measurement formula (IUMF). The detectable minimum concentration was 0.792 pg/ml in experiment.

Serum FSH, LH, E_2, P Assay

EIA (enzyme-labeled immuno-sorbent assay) was applied to measure serum FSH, LH concentration; RIA (radioimmunoassay) was used to determine serum E_2, and 4.2% for serum P; CV within inter-batch were 8.1% for serum E_2 and 7.3% for serum P. Microne enzyme-labeled immunoassay (MEIA) kit, bought from Abbott Lab in America, was applied to measure serum CA-125 concentration, with coefficients of intra-groups and inter-groups variation of 3.16± 1.44% and 5±1.5%, respectively. It was considered abnormally elevated when serum CA-125 level was more than 35U/ml.

Statistical Analysis

Mean values (MVs) of data were expressed by $\bar{x}\pm s$; Unpaired counting materials were analyzed by Student's *t*-test; The differences of sample rates from counting data were analyzed by Chisquare test; Spearman rank correation was applied to analyze the relativity between two parameters.

RESULTS

Serum INH Contents during Follicular, Peri-ovulatory and Mid-luteal Phase in Fertile Women

Serum INH concentrations during follicular, peri-ovulatory and mid-luteal phase were 9.48 ± 7.10 pg/ml (2.04~18.53 pg/ml), 19.04 ± 9.73 pg/ml (3.49~33.26 pg/ml), 131.13 ± 110.81 pg/ml (3.49~341.10 pg/ml), respectively. Mean value of serum INH concentrations was 241.92 pg/ml, during mid-luteal phase was set as upper limit of normal range of serum INH concentration. There was only one case (2.56%) whose serum INH level was over 241.92 pg/ml in controlled group. The mean values of serum INH concentrations during different phases were significantly different, $P<0.01$.

Correlativity between Serum INH and FSH Levels

in Fertile Women

In fertile women, serum FSH concentrations were negatively correlated with serum INH concentrations by Spearman rank correlation analysis, r_s=–0.483, P<0.01. Figure 1 shows the dynamic variation of serum INH and FSH concentrations during menstrual cycle in one 36 year old woman.

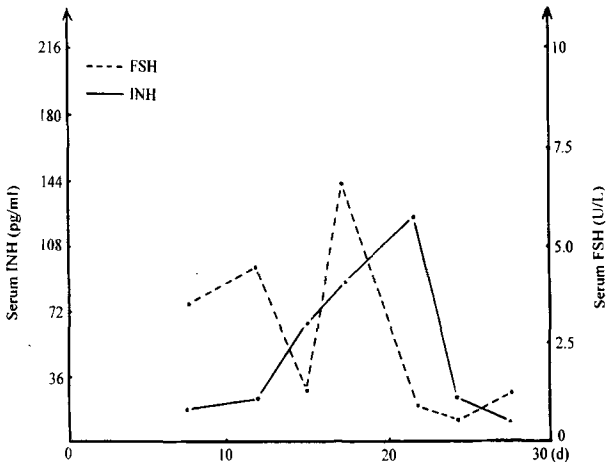

Fig. 1. Relativity between serum INH and FSH concentrations

Serum INH Contents in Menopausal Women

The serum INH levels were undetectable in 9 cases. In the other 7 cases, the mean value of serum INH concentrations was 2.77 ± 1.212 pg/ml (0.792~4.79 gp/ml), and median, or 3.89 pg/ml, was setted as the upper limit of normal range. There was only one case (6.25%) whose serum INH level was above the upper limit in 45 years of age. It was considered to be the cause by active residual ovarian tissue.

Serum INH and CA-125 Concentrations in Patients with Ovarian Cancer

As shown in Table 1, in study group, serum INH concentrations were abnormally elevated in 25 cases (75.8%), and serum CA-125 concentrations abnormally increased in 12 cases (36.4%). There was significant difference between two sample rates, P<0.01. Serum INH values were markedly elevated among women with ovarian granulosa cell tumor, thecoma, mature teratoma and ovarian endometrioid cyst, but their serum CA-125 concentrations were in normal range, the remarkble difference existed between two sample rates, P<0.05. The mean values of serum INH concentrations among ovarian granulosa cell tumor, thecoma, mucinous cystadenocarcinoma and malignant teratoma cases were notably higher than that of other kinds of tumors cases, P<0.01; and that mean values of serum CA-125 concentrations in cases of ovarian serous cystadenocarcinoma and mucinous cystadenocarcinoma were significantly higher than cases of other kinds of tumors, P<0.05. Statistical analysis showed that positive rate of serum CA-125 concentrations for serous cystadenocarcinoma cases was prominantly higher than that of serum INH concentrations, P<0.05; There was no significant difference of positive rate for mucinous cystadenocarcinomsa cases between these two tumor markers, P>0.05. In all ovarian cancer cases, serum INH concentrations fell into normal range one week after operation except one case whose serum INH level still increased above normal range one week after debulking surgery. Spearman rank correlation analysis indicated that there were no correlativity between serum INH and CA-125 values in study group, r_a=0.224, P>0.05.

Table 1. Serum INH and CA-125 concentrations in ovarian cancer women

Histological type	No.	Serum INH (pg/ml)			Serum CA-125 (μ/ml)		
		elevation(%)	mean value	range	elevation(%)	mean value	range
Granulosa cell tumor	3	3 (100)	3716.53	415.30~9348.59	0	30.31	25.76~33.48
Thecoma	2	2 (100)	4674.02	816.23~7183.30	0	25.63	21.78~34.60
Mucinous cystadeno carcinoma	6	5 (83.3)	5160.42	93.78~9499.07	3 (50)	167.99	18.63~600
Serous cystadeno carcinoma	6	2(33)	406.44	75.17~1207.15	5 (83.3)	304.38	33.42~600
Endometrioid carcinoma	2	2 (100)	658.76	500.94~816.55	1 (50)	33.33	25.4~71.34
Malignant teratoma	4	2 (50)	3197.16	66.53~6540.88	2 (50)	54.37	5.47~138.8
Benign teratoma	4	4 (100)	978.48	643.21~1246.21	0	21.7	16.8~28.7
Endometrioid cyst	4	4 (100)	814.32	519.59~1061.75	0	27.6	18.9~32.6
Krukenberg's tumor	2	1 (50)	234.72	99.9~369.50	2 (100)	55.47	43.25~67.68

The Monitoring Effect of Serum INH Content Measurement for Patients with Ovarian Cancer

Case 1: One woman age 47 years with ovarian granulosa cell tumor, left-side adnexectomy was carried out in June 7, 1994, serum INH levels decreased from 531.72 pg/ml before operation to 82.94 pg/ml one week after operation, chemotherapy was not received due to financial reason. She had a complaint of abdominal turgor progressively aggravated since July, 1995. On Oct. 10^{th} in 1995, serum INH concentration reached 415.30 pg/ml, however serum CA-125, AFP, and CEA values were in normal range; Recurrence of ovarian granulosa cell tumor in III stage was identified through the second-look laparotomy; after total hysterectomy and right-side adnexectomy were carried out, serum INH level fell into 3.49 pg/ml; and then decreased to undetectable level after six courses of chemotherapy. CP scheme (CTX+DDP) was applied.

Case 2: Ovarian mucinous cystadenocarcinoma in II_b stage, age 62 years, serum INH concentration reached 9499.07 pg/ml with serum CA-125 level more than 600U/ml, and serum AFP and CEA concentrations in normal range. Cytoreductive surgery was performed on Dec. 7^{th}, 1996, and serum INH level decreased to 1390.75 pg/ml one week later; two courses of chemotherapy were given by CAP scheme (CTX+DDP+Adriamycin). But serum INH contents increased again to 6659.32 pg/ml with normal serum CA-125 level after 2 months, she died 6 months later.

Case 3: Ovarian endometrioid carcinoma in II_b stage, age 42 years, total hysterectomy and both-side adnexectomy were carried out in Sep. 1994; Then after eight courses of chemotherapy by CAP scheme were applied, serum INH concentration rose to 816.55 pg/ml in Aug. 1995, and second-look laparotomy was taken to identify the recurrence of adenocarcinoma in stump of vagina, then cytoreductive surgery was performed, and eight courses of chemotherapy were executed after operation, serum INH concentrations were in normal range from 1.37 pg/ml to 0 pg/ml. She was still alive without any sign of carcinoma.

DISCUSSIONS

INH was mostly synthesized and secreted from ovarian granulosa cells in women. It's molecular structure was constituted from α subunits with different β subunit connected by disulfide bond. In different species, there were same α subunits with different β subunits, which differed from each other with various amino acids only in specific situs; and hereby it was divided into two kinds of subunits, β_A and β_B. Thus there were two forms of INH, INH-A (α-β_A) and INH-B (α-β_B); moreover, free α-subunit (α-C) also existed[4] in circulation. Currently INH immunoassays, enzyme-linked immunoassay INHs established (ELISA) and radioimmunoassay (RIA), isolated from porcine or bovine follicular fluids. The most representative measurement was the Monash method established by McLachlan[5] in 1986. It made research of INH possible in the clinic. The mechanism that polycloning antibody to INH could conjugate with α-subunit was applied in our study. To our knowledge, the modified INH-RIA was first established in our nation. Serum INH concentrations during menstrual cycle in fertile women, menopausal women and ovarian cancer cases were monitored dynamically. Modified INH RIA was identified to be convenient, timesaving, quantitative and especially highly sensitive (<1 pg/ml); it provided favorable methodological fundament for clinical research. The results showed that there were periodical changes of serum INH concentrations in menstrual cycle for fertile women; it's low in follicular phase, and gradually increased with follicular development towards maturation. Serum INH levels rose markedly after ovulation and to the peak value at mid-luteal phase and it decreased rapidly afterwards. This illustrated that serum INH was the staple products of ovarian grnulosa cells in follicular phase, but mainly released from luteal cells in luteal phase. That significantly negative correlations between serum FSH and INH concentrations confirmed that INH could specifically inhibit synthesis and secretion of FSH from anterior pituitary gland, accordant with other reports.[6]

It was reported[7] that no serum INH existed, or unmeasured, in menopausal women. In this study, serum INH concentration was measurable in seven menopausal women. We considered that although ovaries had no function of ovulation in menopausal women, it need rather long periods for them to be completely in loss of ovarian function from the very beginning of menopause period. On the other hand, the diverse results from different studies were probably due to the distinct sensitivity between different method. In all conscience, it stills demands further discussion about whether there were influencing factors of cross reaction in test.

Although there had been various markers for monitoring ovarian cancer, such as: CA-125, CA-153, AFP, ovarian antigen and ovarian carcinoma related antigen and so on, all of that have obvious limitations in view of specificity and sensitivity. For example, serum CA-125 was merely sensitive to serous cystadenocarcinoma. It was deserved to pay attention that INH is not only a kind of indicators for ovarian granulosa cell tumor but also maybe one nice marker for mucinous cystadenocarcinoma.[8] Besides granulosa cell tumor, the results of this study suggested that INH possessed important value in the diagnosis of ovarian malignant tumors (Thecoma, mucinous cystadenocarcinoma, endometrioid

carcinoma) and benign tumors (mature teratoma, endometrioid cyst). The research still found that serum INH concentration was elevated in the case of recurrent ovarian carcinoma after oophorectomy, and meanwhile this finding validated that INH could be produced and secreted in related ovarian tumor tissues other than ovarian granulosa cells and that probably was the biological base for INH to be ovarian tumor marker, despite the fact that the exact mechanism was still subject to in-depth research.

We considered that joint application of INH and CA-125 was likely contributing to early diagnosis, treatment and follow-up for various kinds of ovarian carcinoma and worthy of thorough exploration.

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