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Original Article

Granulosa Cell – Stromal Tumors: An Immunohistochemical Study Including Comparison of Calretinin and Inhibin

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ABSTRACT

Background and Objective: **Histopathological evaluation of granulosa cell tumors (GCT) of the ovary may be confused morphologically with a wide variety of the tumors. Immunohistochemical staining for inhibin and calretinin can be used for better diagnosis. Although it has been suggested that inhibin can be a sensitive marker for GCT, it maybe had negative results in some cases. In addition, caltrinin has been proposed as a marker for GCT. The aim of this study was to investigate the immunohistochemical methods (IHC) including a comparison of calretinin and inhibin markers in the diagnosis of these tumors.**

Patients and Methods: **This prospective study carried out from 2000 to 2009 at Ghaem and Omid hospitals, Mashad University of Medical Sciences, Iran. A total of 23 ovarian GCT specimens were immunostained with commercially available antibodies to find out calretinin and inhibin immunoreactivity. Data were analyzed by descriptive statistical method. A P value of =<0.05 was considered significant.**

Results: **For diagnosing GCT, the sensitivity of calretinin was 100% and that of the inhibin was almost 73.9%. The extent and severity of staining was more extensive for calretinin compared to inhibin *P*<0.001.**

Conclusion: **Calretinin is a more sensitive biomarker for GCT than inhibin.**

Key words: Calretinin, Inhibin, Granulosa Cell Tumor, Immunohistochemistry, Sex Cord-Gonadal Stromal Tumors

Introduction

Ovarian sex -cord stromal tumors are relatively infrequent tumors, accounting for 1% of all ovarian tumors, and granulosa cell tumors (GCT) account for 610%- of malignant ovarian neoplasms (1). Typical histomorphologic findings in GCT are call -exner bodies and coffee bean grooved nuclei. These parameters are not found in all GCT, so we

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must investigate and research other clues for the diagnosis of these neoplasms (2).

Because these tumors are morphologically heterogeneous, they may be confused with a wide variety of the other tumors, such as other ovarian stromal tumors and metstatic carcinoma, so immunohistochemical (IHC) study can be a supplement method to all parallel routine methods used for histological evaluation employed in different diagnosis (3).

Typical features of GCT are hormonal activity; moreover, these tumors often produce estrogen and inhibin. Measurement of inhibin concentration may be useful in the diagnosis of GCT. Inhibin is a hetrodimeric glycoprotein hormone composed of an alpha and beta subunit. Determination of alpha inhibin using radioimmunoassay appears to be a more reliable indicator for presence of GCT (4).

Unfortunately, inhibin is neither completely specific nor sensitive for diagnosing GCT. It may be a negative marker in some of ovarian sex cord stromal tumors and positive in some of other groups of ovarian carcinoma, non -GCT groups (5). Deavers *et al*. reported 2830%- of ovarian stromal cell tumors that have been inhibin positive, whereas inhibin has been demonstrated to be positive in renal cell carcinoma, adrenal cortical carcinoma and clear cell carcinoma of ovary (6).

We must mention that other tumor markers which have been used to improve the diagnosis of GCT, included relaxin –like factor melan-A, mullerian inhibitory substance and calretinin (7).

Calretinin is a calcium binding protein, which is expressed primarily by selected neurons in peripheral and central nervous system. It has been found in mesothelial cells and mosotheliomas(8). In ovary, calretenin was expressed in the theca internal cells, leydig cell tumor, hilus cells and scattered individual stromal cells. It has been reported that calretinin is expressed by GCT .Using a monoclonal antibody anticalretinin can be used to investigate the expression of calretinin by IHC staining. Generally, this can be useful for establishing the diagnoses in problematic GCT cases (8, 9).

Occasional serous papillary tumor of ovary demonstrated calretinin and inhibin staining and poorly differentiated carcinoma were inhibin positive; it should be kept in mind that both inhibin and calretinin will stain a small proportion of endometriod ovarian carcinoma (8).

One study showed strong positive staining of granulosa cell in follicular cysts and corpra luted. There was also positive staining in hyperthecosis and strumal hyperplasia of ovary (5).

It was demonstrated that both inhibin and calretinin were useful in the diagnosis of GCT (10), but another research revealed that calretinin was present in 690%- of GCT whereas inhibin reactivity ranged from 590%- of these tumors (11). Review of literature revealed that calertinin cannot replace but could complete IHC panel, which is used for diagnosis of GCT (12).

Because of confusing morphologically in our cases, we investigated an IHC study including a comparison of calretinin and inhibin for diagnosing GCT.

Material and Methods

In this cross-sectional descriptive retrospective study, from 2000 to 2009, 23 patients who had ovarian granulosa cell tumors and referred to Ghaem and Omid hospitals of Mashhad University Medical Sciences, Iran, were selected.

Besides, tumors of these patients as determined by H&E staining and conventional microscopic examination were investigated thoroughly by pathologist. All these tumors underwent additional sectioning in four thick micrometers and staining with H&E and an immunohistochemical stain, were carried out.

Moreover, immunoperoxidise streptoavidin-biotin procedure was performed. After deparaffinization and hydration, slides were incubated with hydroxiperoxidese. Antigenic retrieval was carried out by incubation with molar citrate buffer %1 (pH=6) in microwave oven for 20 min .Then slides were incubated with antibody, calretinin and inhibin α for 60 min in normal room temperature .

In the next stage biotinylated link antimouse and antirabbit immunoglobulin and streptoavidin peroxidase (DAKO LSAB 25 System , peroxidase kit , Denmark) was used . Peroxidase was shown with diaminobenzidin hydrochloride (DAB), counter

staining was established with hematoxyline mayer and mounted in Canada Balsam.

For negative control, inhibin –α and calretinin were omitted in this process and for positive control of calretinin mesothelioma and for inhibin –α testis tissues that had been selected were used. After that, immunohistochemical staining with special antibodies was performed and tissue specimens were evaluated for inhibin-α and calretinin by pathologist. The severity staining was evaluated on a 04- scale based on a percentage of neoplastic cells labeling 0=none;1=125%- ; 2=2650%- ; 3=5175%- ; 4=76-100%

Results

Among 23 tissue samples with histopathologic diagnosis of granulosa cell tumor of ovary, 100% were calretinin positive and 73.9% were inhibin-α positive. So positive staining as a method for diagnosing for calretinin was significant (fisher's exact test) ($P \leq 0.022$) (Fig. 1).

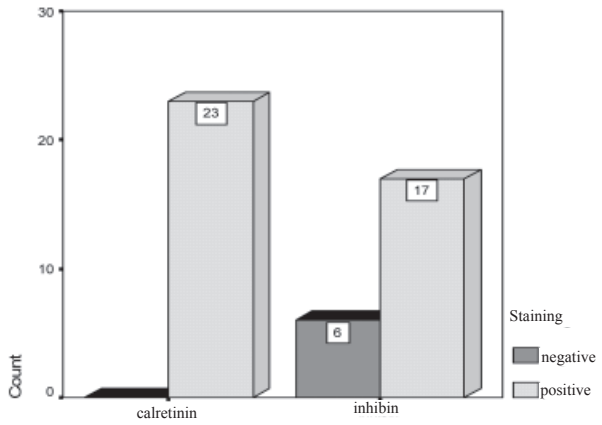

Fig. 1 : Result of immunohistochemical staining of granulosa cell tumor specimens for calretinin and inhibin

The severity of staining was evaluated by the percentage of cells that absorbed the stain. The stain absorption for calretinin was 7022.7%+ averagely and for inhibin was 33.531.8%+ averagely; therefore, there was a significant difference between staining severities for these two biomarkers (mannwhitney U test, $P \leq 0.001$).

Descriptive report of staining indicated significantly stronger staining for calretinin compared to inhibin (Table 1).

Table 1 : Severity of immunohistochemical staining of granulosa cell tumor specimens for calretinin and inhibin

Staining severity	Calretinin	n(%)	Inhibin	n(%)
Negative	0	(-)	6	(26.1)
Weak	0	(-)	1	(4.3)
Moderate	5	(21.7)	10	(43.5)
Severe	18	(78.3)	6	(26.1)
Total	23	(100)	23	(100)

The extent of staining was also assessed and was significantly more extensive stain absorption was observed for calretinin compared to inhibin (Fisher,s exact test, $P \leq 0.001$) (Table 2).

Table 2: Extent of immunohistochemical staining of granulosa cell tumor specimens for calretinin and inhibin

Staining Extent	Calretinin	n (%)	Inhibin	n (%)
0	0	(-)	6	(26.1)
1+	0	(-)	5	(21.8)
2+	5	(21.7)	6	(26.1)
3+	8	(34.8)	3	(13.0)
4+	10	(43.5)	3	(13.0)
Total	23	(100)	23	(100)

Finally in order to diagnose granulosa cell tumor, the sensitivity of calretinin was 100% and sensitivity of inhibin was almost 73.9%, according to these data calretinin was a high sensitive biomarker for the diagnosis of ovarian granulosa cell tumors (Fig. 2-4).

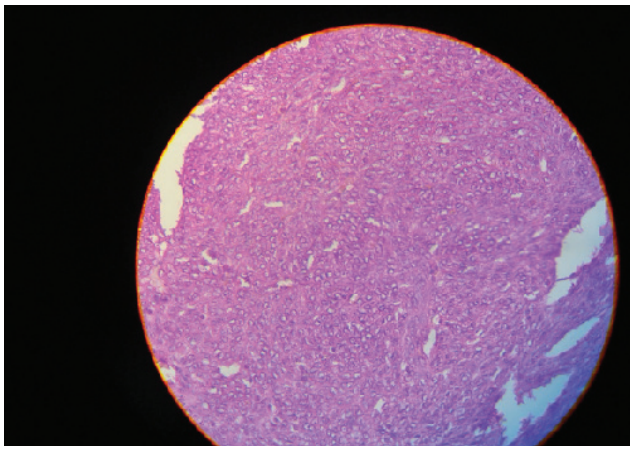

Fig. 2 : Microscopic view of ovarian granulose cell tumor (H&E ×400)

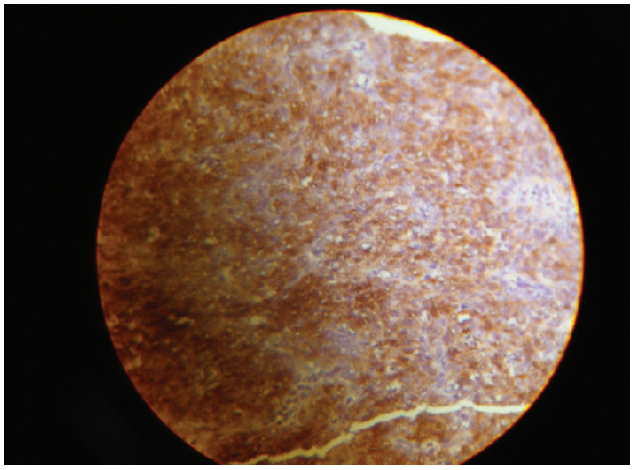

Fig. 3 : Diffuse positive immunoreactivity for calretinin marker (IHC staining)

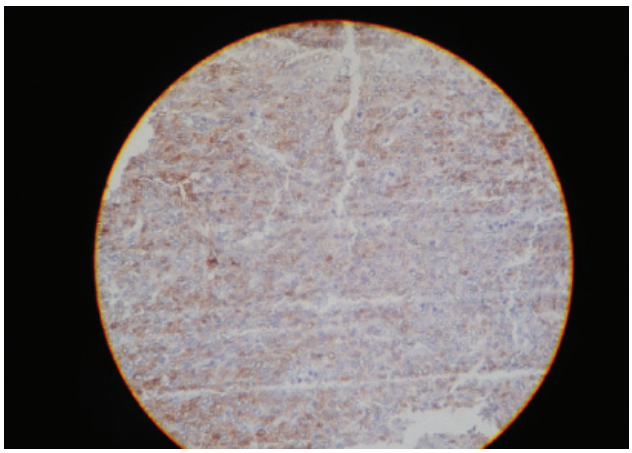

Fig. 4 : Focal immunoreactivity for inhibin marker (IHC staining)

Discussion

Granulosa cell tumors are rare neoplasms of ovary composed of the cells with the morphology of hormone production cells , but hormonal study for GCT diagnosis is less helpful .One of the most useful tests is immunomarkers assay for the diagnosis of GCT, therefore we must investigate other possibilities (2).

Macroscopic features of GCT are usually encapsulated with a smooth, lobulated outline and a predominantly solid cut surface. Microscopic appearance of GCT is extremely variable and its pattern of growth includes micro follicular, trabecular, insular, solid and diffused sarcomatoid forms.

IHC staining of inhibin, although generally useful in the diagnosis of GCT, is not positive in every case and, so we must weigh the possible alternatives and use of other markers (13). Calretinin staining will aid us in different levels of diagnoses for differentiating GCT from the others undifferentiated carcinoma (14).

We undertook this study to evaluated the potential utility of calretinin in diagnosis GCT and to compare it with inhibin which expressed by these tumors. In our study, calretinin was detected in 100% of GCT cases whereas inhibin was identified in 73.9% of these specimens, for our survey offers calretinin marker better than inhibin with more sensitivity in ovarian GCT diagnosis. These results were defined in an identical manner of previous studies (15). A wide range of studies of both calretinin and inhibin expressions illustrated uniform results, (calretinin positive 98% vs. inhibin positive 95%) (16).

In one study, all six cases of GCT were positive for both calretinin and inhibin. However, calretinin was expressed in a grater percentage of tumor cells than inhibin in each case. The consistent diffuse staining for calretinin compare with the variable staining for inhibin suggested that calretinin may be the more sensitive of the two for diagnosis GCT(6). Although in other survey, the sensitivity of calretinin versus inhibin for the entire group of GCT was identical at 56% (16, 17).

The limitations of our study were due to two factors. The first one was multiple-step because of the sensitivity of the study and the second factor was the rarity of this tumor, which resulted in the difficulties in pathological specimen that led to laboratory-based problems.

We believe that according to variable morphology

and the behavior of GCT it is needed an IHC study for differential diagnosis from other neoplastic lesions for establishing definite diagnosis of challenging cases

Conclusion

We recommend that calretenin cannot replace but will be able to use as a complement of inhibin as part of an IHC panel used for diagnostically challenging cases. Further research studies with larger sample size are suggested.

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