Alteration of the K-Ras Gene Expression in Atypical and Nonatypical Hyperplastic Endometrium

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Abstract

Background: Endometrial cancer is the most common gynecologic malignancy that has often proceeded by a premalignant phase. Modern molecular and immunostaining methods for precancerous lesions diagnosis have been expanded. One of the genetic alternations in the endometrial cancer carcinogenesis is the mutational activation of the K-ras oncogene. K-ras mutation has recognized to occur at an early stage of neoplastic progression in the endometrium. The purpose of this study is to investigate the expression pattern of K-ras gene in atypical and nonatypical hyperplastic endometrium.

Methods: In a prospective study in the referral gynecologic hospital in Tehran, immunohistochemical evaluation of K-ras has performed on 72 consecutive specimens in two following groups: endometrial hyperplasia without atypia (n: 36), and endometrial hyperplasia with atypia (n: 36). Staining of cells has evaluated in arbitrary quantitative methods in regards to both slides area staining and intensity of color reaction.

Results: K-ras immunoreactivity has seen in 3/36 (8.3%) cases of non atypical hyperplasia and in 2/36 (5.6%) cases of atypical hyperplasia (P: 0.64).

Conclusion: We have not establish any significant differences in K-ras expression between the atypical and nonatypical hyperplastic endometrium, and our data has supported this view that K-ras mutation is a very rare event in human endometrial carcinogenesis.

Keywords: K-ras; Endometrial cancer; Endometrial hyperplasia; Immunohistochemistry

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Introduction

Endometrial carcinoma is the most common cancer in female genital tract in many industrialized countries and its most frequent subtype, endometrioid endometrial carcinoma, is often preceded by a precursor lesion [1].

According to the biological and clinical features endometrial carcinoma has been classified into two types [2]. Type 1, those includes low grade endometrioid endometrial carcinoma, comprise 70 to 80% of sporadic endometrial carcinoma [3]. They have associated with estrogen stimulation and often preceded by endometrial hyperplasia [3, 4]. In contrast, type II endometrial carcinoma consists of high grade carcinoma of non endometrioid differentiation, usually papillary serous, then the second frequent one, clear cell with an aggressive clinical course [2]. Endometrial carcinoma has characterized by alteration in various molecular genetics. Multiple molecular studies have analyzed endometrial carcinoma for this alteration by various molecular markers. Mututions of PTEN and K-ras are the most widely reported mutations within premalignant endometrium [4]. The most frequently altered gene in endometrioid endometrial carcinoma is PTEN, which up to 83% of endometrioid endometrial carcinoma and 55% of precancerous lesions show loss of PTEN expression [2]. The ras gene family consists of three well characterized genes, K-ras, H-ras and N-ras. The K-ras proto-oncogene has encoded a protein (p21-ras), belonged to the family of GTP/GDP-binding proteins with GTPase activity [5] then encoded small inner plasma, cellular membrane GTpase, which made as a molecular switch during cell signaling. Therefore this gene is largely related to tumor growth and differentiation [6, 7]. Ras mutations could be the most common oncogenic mutations which

have detected in human malignancies, but the incidence of K-ras activation varies widely among carcinoma. The implication of K-ras mutations in colorectal carcinoma and other types of human cancer is common in the literature [5]. In fact, exon 2 is the hot-spot mutation region for the K-ras gene and the most frequent alterations are detected in codons 12 (about 82% of all reported K-ras mutations) and 13 (about 17%) [5]. K-ras mutations have been reported in 19-46% of endometrial carcinoma. These mutations mostly have seen in type I tumors and reported in 10-30% of cases of endometrioid endometrial carcinoma, where as they have never detected in papillary serous and clear-cell carcinoma [1, 6, 8]. Mutations are also detected in 4.5-23% of endometrial hyperplasia, suggesting that mutations in the K-ras gene may present and early event in tumor genesis within the subset of type I endometrial carcinoma [2, 9]. Some studies have demonstrated that the frequency of K-ras mutations have raised progressively from simple hyperplasia, to the complex hyperplasia, then to the carcinoma, and detection of K-ras mutations in pre-malignant biopsy samples has suggested as a progression marker to malignancy [6]. For investigating K-ras mutation, K-ras codons 12 and 13 point mutations have examined by direct sequence analysis, where as the ras p21 expression has evaluated using immunohistochemistry. Immunohistochemistry has widely used to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue, then could provide change evidence in gene activities, and it is simpler to perform as well as colony construction and mutational assays [5, 10]. The first practical immunohistochemical marker for premalignant endometrioid endometrial carcinoma is loss of the PTEN protein that could enable detection of PTEN-null endometrial glands [11].

In the most previous studies K-ras gene mutation has mainly assessed by PCR, other than in present study we have used immunohistochemistry method to evaluate K-ras expression in two groups of specimens, from the nonatypical hyperplasia and atypical hyperplasia of endometrium.

Materials and Methods

This prospective study has performed in Women Hospital, a referral gynecological hospital in Tehran. Eligible specimens have enrolled from consecutive population of endometrial curettage, then reviewed and selected, could presented by an expert gynecology pathologist (NIM). The study has approved by a university ethical committee.

Tissue specimens including: 36 cases of nonatypical hyperplasia of endometrium; that has consisted of 28 cases of simple hyperplasia and 8 cases of complex hyperplasia and 36 cases of atypical hyperplasia; consisted of 3 cases of atypical simple hyperplasia and 33 cases of atypical complex hyperplasia. Diagnosis of hyperplasia was according to WHO histological classification, (WHO 94) [12]. Specimens with any evidence of chronic nonspecific endometritis, endometrial polyp, secretory changes or progesterone effect have excluded.

Immunohistochemistry: one paraffin block has selected from each case and cut at a thickness of 4µm. The sections have deparaffinized in xylene and dehydrate through a series of graded alcohols. Antigen retrieval has achieved by heat treatment at 98° centigrade's, in PT module buffer 1(citrate buffer, PH=6.0), for 20 minutes. Endogenous peroxides activity has blocked by incubating slides in serum blocking solution. Then the sections have incubated with Rat anit K-ras primary antibody (polyclonal antibody from Zymed laboratories, South San Francisco, CA, USA) that has previously diluted 1/100 for 45 minutes. The reaction has visualized with the Zymed immunohistochemical detection kit using Diamminobenzidine (DAB) chromogene as substrate. Finally, the reactions have counterstained with Mayer's hematoxylin. Tissue sample from adenocarcinoma of colon has used as positive control and the negative control has run without primary antibody addition. Immunohistochemical staining result has evaluated synchronous by two pathologists. Immunoreactivity has regarded as positive when brown staining has localized in the nuclei or cytoplasm of hyperplastic endometrial glandular cells and graded arbitrarily and semi quantitatively by considering the percentage and intensity of staining on whole of the section. Staining of cells has scored as 5-10%, 10-50%, 50-70% and >70% of stained slides area. Analyses have carried out by SPSS (software Chicago, IL, USA) version 15, using chi-square tests. P value <0.05 has considered significant by one-way ANOVA.

Results

K-ras immunoreactivity has noted in 3 cases of 36 nonatypical hyperplasia (8.3%) and in 2 cases of 36 atypical hyperplasia. The difference of immunoreactivity between two groups was insignificant (P: 0.0643 by one-way ANOVA). The percentage of stained slides area in 3 cases of nonatypical hyperplasia was 0-10% and also in 1 case of atypical hyperplasia. One case of atypical

hyperplasia has shown 50-80% stained slide area. But the intensity of staining in all cases was weakly, and there was no significant difference between nonatypical and atypical hyperplasia (P: 0.365 by one-way ANOVA). The K-ras immunoreactivity was heterogeneous. Some cells within a gland or some glands were positive for K-ras staining especially in nonatypical hyperplasia. The mean age of nonatypical and atypical complex hyperplasia was 45.9 and 47.4 years respectively and the mean age of negative and positive K-ras expression was 46.2 and 52.2 years respectively (P: 0.140 by one-way ANOVA), without any significant differences.

Discussion

Endometrial carcinoma has risen theoretically from a series of somatic mutations, which alter cytology and architecture of benign endometrium, to expanded less differentiated histological lesions.

Genetic and endocrine abnormality mechanism has been integrated into a multistep model for carcinogenesis [13]. Because of the problems in recognition of possible premalignant endometrial lesions, based on their histologic feature alone, there was a significant need for markers to distinguish early neoplastic lesions in the endometrium, that could be a really high risk of progression to carcinoma, from those ones that might spontaneously regress, or those might respond hormonal therapy.

PTEN inactivation is the most frequent genetic alternation in endometrial tumors [14]. K-ras proto oncogene has largely related to tumor growth and differentiation; and mutations of the K-ras were present in about 10-30% of endometrial carcinoma [2].

K-ras mutations in 16% of the cases of endometrial hyperplasia have indicated that it might be present on early event within a subset of endometrial carcinoma [2]. K-ras and PTEN mutations have not seemed to concur within the same tumor [2, 15]. In current study, we have focused on the analysis of K-ras gene expression in patients that had endometrial hyperplasia, but not yet among the malignant transformation of the endometrium mucosa. Our work has designed to detect k-ras protein expression by immunohistochemistry method in nonatypical and atypical hyperplasia. Immunohistochemistry has regarded as cost saving, not specific for codons and a preliminary result.

In literature review, we have not found any article in relation to evaluation of k-ras expression by immunohistochemistry in endometrioid endometrial carcinoma. Just in one article, by Elsabah et al, immunohistochemical assay for detection of k-ras protein expression in metastatic colorectal cancer has evaluated and they have concluded immunoreactivity, may be compliment PCR in the detection of K-ras mutation [10].

As reported previously, we have detected K-ras immunoreactivity in 3 cases (8.3%) of nonatypical hyperplasia which were one simple hyperplasia and two complex hyperplasia.

There is no significant difference in immunoreactivity between two types of hyperplasia.

Matias et al reported K-ras mutations in 18.9% of their endometrioid endometrial carcinoma but they have detected K-ras mutations in only one of 22 (4%) of endometrial hyperplasia. In this case atypical hyperplasia has coexisted with endometrioid endometrial carcinoma [16]. Also in another study has done by Enomoto et al, K-ras mutation has found in 2 of 16 hyperplasia histologically classified as atypical hyperplasia and non of 6 adenomatous hyperplasia and non of 12 cystic hyperplasia have contained any detectable K-ras mutations [17]. In our study only 2 cases (5%) of atypical hyperplasia, both atypical complex hyperplasia show immunoreactivity for K-ras, and also the difference of K-ras immunoreactivity between nonatypical hyperplasia and atypical hyperplasia was not significant. Hongbo et al has found K-ras mutations in 3 of 13 monoclonal atypical complex hyperplasia (23%) but not in 2 polyclonal atypical complex hyperplasia or in 31 hyperplasia without atypical. They have concluded that K-ras mutation has significantly and frequently found in endometrial hyperplasia with atypical, more than those without atypical, and suggested that K-ras activation has associated with the presence of cytologic atypical of endometrial hyperplasia [18].

We propose a concept of endometrial hyperplasia based on the results of clonally analysis would contribute to the pathways of endometrial carcinogenesis. This benign neoplastic process might result from genetic alternation such as activation of proto-oncogens-Kras. The aim of this study was to detect activated K-ras by immunoreactivity method, a routine ancillary method in many laboratories, in non atypical hyperplasia and atypical hyperplasia. But we haven't got significant results. In approximately all of the studies about K-ras expression, which have done by PCR method [18-20], an expensive and unusual method in many laboratories, and therefore our study is the only study that has done by the immunoreactivity method.

Unfortunately, our results about K-ras expression by immunoreactivity haven't confirmed by other

studies about K-ras expression in atypical complex hyperplasia that have done by PCR method.

Conclusion

Our data have indicated that K-ras activation by point mutation could not spotted by immunoreactivity method in early detection of development of endometrioid endometrial carcinoma.

Acknowledgment:

This article was prepared based on doctoral thesis approved by the Medical Ethics and research Office at the Tehran University of Medical Sciences.

Conflict of Interest

The authors have no conflicts of interests in this article.

Authors' Contribution

Narges Izadi-Mood has designed the study, signed H&E and IHC stained slides and analyzed the data. Soheila Sarmadi has signed H&E and IHC stained slides and contributed to the literature review and writing-up process. Behzad Rostamnasl has gathered data and contributed to the IHC staining process.

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