P53 Expression in Endometrial Hyperplasia and Endometrial Carcinoma

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ABSTRACT

Background and Objectives: Mutations of the P53 tumor suppressor gene and alterations in its protein expression often occur in a variety of human malignant tumors, including endometrial carcinoma, but the practical implications of this phenomenon are yet to be fully exploited. This study was designed to evaluate P53 protein expression in normal, hyperplastic and malignant endometrium by immunohistochemical study and to correlate P53 expression in endometrial carcinoma with other clinic-pathological prognostic parameters (age, histologic type, tumor grade, cervical & myometrial invasion, and tumor stage).
Methods: The studied samples included 100 formalin fixed, paraffin embedded endometrial tissue specimens which were divided to the following diagnostic categories: - Proliferative endometrium (n=10); secretory endometrium (n=10); simple hyperplasia (n=10); complex hyperplasia without atypia (n=20); atypical complex hyperplasia (n=10) and endometrial carcinoma (n=40).
Results: None of the normal endometrium, simple hyperplasia and complex hyperplasia without atypia showed P53 immunostaining, while 20% of atypical complex hyperplasia and 32.5% of endometrial carcinoma showed immunoreactivity for P53. In endometrial carcinoma, significant correlation was observed between P53 expression and age at diagnosis, histological grade,FIGO stage, myometrial invasion & cervical invasion ; but not with the histological type .
Conclusions: The results indicated the validity & simplicity of the application of immunohistochemistry in determining the status of P53 overexpresion which is strongly associated with endometrial carcinoma aggressiveness and high malignant potential.

Key words: Endometrial carcinoma, endometrial hyperplasia, p53gene.

INTRODUCTION:

During the last decades, a change of demographics has occurred. The number of women more than 60 years of age is increasing worldwide, with increasing life span. As a consequence, the focus for the aging woman will be on lifestyle support to counteract various features of degenerative changes. This will be accompanied by an increasing demand for medical expertise on lifestyle drugs, for example, the use of hormone replacement therapy. In addition, there is an increase in malignant diseases worldwide, involving women. All these factors might also be associated with a potential increase in endometrial malignancies and their precursors[1]. Endometrial cancer (EC) is the most common malignancy of the female genital tract in the developed countries; it accounts for about 7% of all malignancies occurring in women[2]. However, incidences throughout different regions of the world vary considerably.

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Compared to Africa and Asia , which have the lowest rates of incidence, Western Europe, USA and Canada are shown to have the highest incidence worldwide[3]. Ninety-seven percent of all uterine cancers arise from the glands of the endometrium and are known as EC, the remaining 3% are sarcomas. Hyperplastic abnormalities of the endometrium are potentially a preneoplastic and embrace a spectrum of morphologic disorders, ranging from an increase in the density of glands and their cystic enlargement (simple hyperplasia) to a varying degree of abnormalities in the configuration and distribution of the glands (complex hyperplasia), that are commonly associated with nuclear abnormalities (atypical hyperplasia). The progression to adenocarcinoma is related to histologic complexity and atypicality of endometrial hyperplasia[4]. Recent advances in molecular biology have led to the concept that carcinoma arises from the accumulation of a series of genetic alterations involving activation of proto-oncogenes and inactivation of tumor suppressor genes. The p^{53} gene is one of the most important tumor suppressor genes that is located on the short arm of chromosome 17 and encodes for a 53-kD nuclear phosphoprotein that is expressed in most cells[5]. This gene is important for several reasons including:

a- genomic stability
b- transactivation of genes involved in cell cycle regulation
c- DNA repair
d- apoptosis
e-effectiveness of chemotherapy.
f- prognosis of the disease [6]. Mutation of the p^{53} gene has been reported in a variety of human malignant tumors and is frequently associated with overexpression of p53 protein. The extended half-life of mutant p53 protein allows it to accumulate to high concentration within the nuclei of affected cells, and it can be readily detected by immunohistochemistry with antibodies specific to the p53 protein. However, in normal cells, p53 protein has a short intracellular half-life and such a low steady-state level is usually not detectable by immunohistochemistry [7,8]. p^{53} mutations are believed to be involved in tumorgenesis and/ or tumor progression and have been reported to be associated with the aggressiveness or poor prognosis of many human neoplasms. The immunohistochemical reactivity for p53 protein has been demonstrated to be associated with poor prognosis in many tumors [9]. The aims of this study were to evaluate p53 protein expression in normal endometrium, endometrial hyperplasia and endometrial carcinoma by immunohistochemical technique and to study the correlation between p53 protein overexpressions in endometrial carcinoma with other clinico-pathological prognostic parameters in order to identify high risk group of patients with endometrial carcinoma.

MATERIALS AND METHODS:

The materials used in this study were consisted of 100 formalin-fixed, paraffin-embedded endometrial biopsy specimens, selected from the pathological files of histopathological department of Rizgary Teaching Hospital, Maternity Hospital and some histopathological private laboratories in Erbil city (Kurdistan of Iraq), during the period Jan.2005-July2008. The studied cases were divided into the following groups: Proliferative endometrium (n=10), secretory endometrium (n=10), endometrial hyperplasia (n=40; 10 simple hyperplasia; 20 complex hyperplasia without atypia and 10 complex hyperplasia with atypia) and EC (n=40; 37 endometrioid carcinoma; 3 adenosquamous carcinomoa). The endometrial carcinoma specimens include 9 endometrial curettings tissue and 31 hysterectomy specimens. Histopathological gradeing of carcinoma were re-evaluated according to Ineternational Ferderation of Gynecology and Obestetrics (FIGO) grading system.For all hysterectomy specimens, the existing slides stained with H&E were reviewed and the following parameters were evaluated: histological type of carcinoma, depth of myometrial invasion,

presence of cervical involvement and surgical stage. The stage of disease was determined by using the surgical staging system of FIGO. Myometrial invasion was analyzed using three categories: no invasion, invasion into the inner half of the myometrium and invasion into the outer half of the myometrium. The cervical involvement was registered as either present or absent. The Dako Cytomation EnVision®+Dual link system-HRP(DAB+) Staining protocol was used for immunostaining to detect p53 expression and was applied to formalin-fixed, paraffin embedded tissues. Negative controls, in which N-universal negative control replaced the primary antibody, were run with each batch of stain and positive control tissue specimens for p53 were prepared using breast carcinoma specimens and it was run with each batch of stain. Positive expression of p53 in light microscope, gives clear cut nuclear staining of brown color. Positive cells were determined by counting 1000 tumor cells in the high power feild of light microscope. All significantly stained cells were considered positive and divided by 10 to acquire the percentage (p53 index); at least 10 HPFs were measured for each case for the purpose of scoring. The extent of p53 immunostaining was assessed as follows [8,19]:
- Negative: when p53 index was <5%.
-Weak positive: when p53 index was ≥5% and <50%.
-Strong positive: when p53 index was ≥50%.
P53 score of ≥50% was regarded as overexpression of p53 protein. Statistical analysis was done by using the chi-square (X^2) test in statistical package for social sciences (SPSS) version 11. P values <0.05 were considered statistically significant.

RESULT:

Specific staining with mouse monoclonal anti p53 antibody DO-7 was exclusively confined to the nuclei. The distribution of p53 indices in this study was bimodal, with most cases having negative or a very low p53 index (<5%) or having a very high p53 index(≥50%). None of the normal endometrium, simple hyperplasia and complex

Table 1: P53 protein overexpression in endometrial carcinoma and its relation to common clinicopathologic variables

Clinicopathologic variables	No. (%)	p53 positive No. (%)	*p* value
All cases	40	13 (32.5)	-
Age			
< 60	18 (45)	2 (11.1)	0.009
≥ 60	22 (55)	11 (50)	
Pathologic type			
Endometrioid carcinoma	37 (92.5)	12 (32.4)	0.974
Adenosquamous	3 (7.5)	1 (33.3)	
Histologic grade			
Grade I	24 (60)	2 (8.3)	< 0.001
Grade II	13 (32.5)	9 (69.2)	
Grade III	3 (7.5)	2 (66.7)	
*Myometrial invasion			
< ½	19 (61.3)	1 (5.3)	<0.001
≥ ½	12 (38.7)	8 (66.7)	
*Cervical invasion			
Negative	23 (74.2)	3 (13)	< 0.001
Positive	8 (25.8)	6 (75)	
*FIGO stage			
I	23 (74.1)	3 (13)	0.003
II	6 (19.4)	4 (66.7)	
III	2 (6.5)	2 (100)	

*percentage (p53 index); at least 10 HPFs were

hyperplasia without atypia showed P53 immunostaining, while 2 out of 10 cases (20%) of atypical complex hyperplasia and 13 out of 40 cases of (32.5%) of endometrial carcinoma showed immunoreactivity for p53. (Table 1)

DISCUSSION:

Studies examining the molecular and genetic basis of endometrial carcinogenesis are few compared to studies of other common types of carcinoma. *P53* is described as "the guardian of the genome" as it prevents proliferation of cells bearing damaged DNA[10] and has become a most prominent protein in cancer research[11]. Mutation of the *P53* gene in EC has been demonstrated and it has been suggested that inactivation of the *P53* gene is implicated in carcinogenesis but usually as a late event in the development of EC. Over expression of p53 protein (implying the presence of a *P53* gene mutation) has been demonstrated in approximately 90% of serous carcinoma, while the prevalence of p53 over expression in endometrioid carcinoma is highly variable among different series, ranging from 5.1-61 %[5,8,19-26,28]. This discrepancy in the results could be due to the choice of methods, sensitivity and specificity of different antibodies used, and various interpretations of the results. There are accumulative evidences that immunohistochemical staining for p53 can identify patients with EC who have an adverse outcome. Although immunohistochemical detection of p53 in sections correlates well with *P53* gene mutations, the concordance between p53 overexpression and gene mutation is not 100% i.e. nuclear *P53* overexpression could sometimes occur in the absence of mutation detected by PCR and vice versa. In a large series of human carcinomas, soong *et al*[12], compared immuno-staining and mutational analysis of *P53* gene, they found discordant results with the two techniques in 24% of 122 EC cases; they did not specify cell type of the EC, but based on other studies it is clear that p53 over expressing EC is of serous type that contains p53 mutations in most cases[13]. The situation for p53 over expressing EC of endometrioid type is less clear. Stewart *et al* [14] did not find any *p53* mutation in 18 endometrioid carcinomas overexpressing p53; however, in most of the cases in their study (14out of18), p53 over expression was focal. In contrast, Lax *et al* [15] found that 87.5% of endometrioid carcinoma in which more than 50% of tumor cells expressed p53 contained *p53* mutation, whereas only 13.3% of endometrioid carcinoma, in which 10-50% of the tumor cells expressed p53, contained p53 mutation. The reason proposed for identifying a p53-positive phenotype by IHC without detection of *P53* gene mutation included alterations in genes other than p53 in its pathway(such as MDM) [16] leading to accumulation of wild type p53 protein , and DO-7 recognizes both mutant and wild type p53 forms. The explanation described for the presence of mutation in the absence of p53 overexpression is the deletion mutations or nonsense mutations which result in stop codons or production of truncated proteins that are not detectable by IHC[17]. However, in comparison with DNA sequencing, IHC methods are cheaper, easier and more familiar to the pathologist as a standard procedure in everyday diagnosis. In the current study, none of the normal endometrium, simple hyperplasia, and complex hyperplasia without atypia showed any positivity for p53. These negative results were also reported by Elhafey *et al,* [18] and Sakuragi *et al,* [19]. P53 immuno positivity was observed in 2 out of 10 cases (20%) of atypical endometrial hyperplasia, Figure(1). Elhafey *et al* [18] found immuno positivity in 3 of 10 cases (30%) of atypical endometrial hyperplasia, while Sakuragi *et al*[19] found no p53 immuno positivity in 3 cases (100%) of atypical endometrial hyperplasia. This variability can be explained by the small sample size of atypical endometrial hyperplasia. P53 immuno positivity in EC cases was observed in 13 out of 40 cases 32.5%) (Table1), a figure lying within the range of previously reported immunohisto-

chemical studies of p53 in type 1 EC (5.1-61%)[8]. This result is comparative to that reported by Fernando *et al* [5]; Kounelis *et al*, [20]; Veral *et al*, [(21)] who reported a positive frequency of 32%, 35%, and 34% respectively. In contrast Erdem *et al* [(22)]; Egan *et al* [23]; and Jeon *et al* [24] who reported lower positive results of 19.2%, 5.1% and 20.4% of cases respectively. While Geisler *et al*[25] and Ragni *et al*[26] reported higher positive results of 57.3% and 61% of cases respectively. Age at diagnosis of EC was correlated with p53 expression. In p53 positive group, the percentage of patients with age above 60 years (50%) were significantly more than patients with age below 60 years (11.1%)(Table 1). Our finding agrees with that obtained by Neilsen and Nyholm[27)] but disagrees with that obtained by Fernando *et al* [5]. Etebary *et al*[6] found similar results in breast cancer, so this data support that p53 mutation frequently occur in older patients than young. The correlation between p53 expression and tumor grade was highly significant (p<0.001); 8.3% of grade I, 69.2% of grade II, and 66.7% of grade III showed p53 immuno positivity (Table 1) and (Figures 2-4). These results are comparable with those obtained by Gassel *et al* [30], Geisler *et al* [25]; Fernando *et al* [5]; Kounelis *et al* [20]; Maeda *et al* [28]; and Erdem *et al* [22]. In contrast, no such correlation was found in studies obtained by Kohlberger *et al* [29] and Ragni *et al* [26]. A highly significant relationship was observed between p53 expression and myometrial invasion (p<0.001); p53 expression was observed in 66.7% when there was deep myometrial invasion and 5.3% when there was superficial myometrial invasion (Table 1). These results are closely comparable with those obtained by Hamel *et al* [31] Fernando *et al*, 2000 [5]; Ohkouchi *et al* [32] and Jeon *et al*[24]; but disagrees with those obtained by Erdem *et al* [22]. Ragni *et al* [26] and Engelsen *et al* [33]. A significant correlation was observed between p53 expression and cervical invasion in the present study (p<0.001); 75% of cases showed p53 expression when there was positive cervical invasion compared with 13% p53 expression in those with no cervical invasion (Table 1). These results are in agreement with those obtained by Kohler *et al*, 1992[34], but disagree with that obtained by Fernando *et al*, 2000[5].The other significant correlation was observed between p53 expression and FIGO stage of EC (p=0.003); 13% of stage I, 66.7% of stage II, and 100% of stage III tumor showed p53 immuno positivity (Table 1). These results are in agreement with those obtained by Geisler *et al* [25],Kounelis *et al* [20] Ohkouchi *et al* [32] and Jeon *et al* [24] but disagrees with those obtained by Erdem *et al* [22] and Ragni *et al* [26].No significant statistical correlation was found between p53 expression and histological types of EC (p=0.974); 12 out of 37 cases (32.4%) of endometrioid carcinoma and 1 out of 3 cases (33.3%) of adenosquamous carcinoma showed positive p53 immunostaining (Table 1), this may be due to small sample size of adenosquamous carcinoma versus endometrioid carcinoma in the current study. Our results are in agreement with Jeon *et al* [24] , but disagree with other studies which showed a significant correlation of p53 expression between endometrioid and non endometrioid type of EC as Erdem *et al*[22] Egan *et al* [23] and Ragni *et al* [26]. In conclusion, P53 mutation seems to have an important role in the carcinogenesis of EC. Considering the acceptable reliability and feasibility of IHC method for detection of P53 mutation, this technique may be expected to serve as a new genetic marker for predicting recurrence and response to chemotherapy in patient with EC.

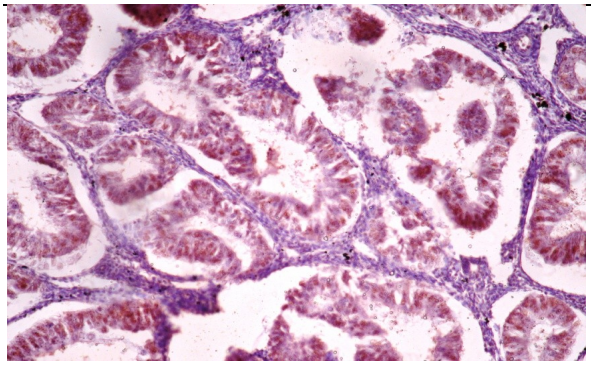

Figure 1: Atypical complex hyperplasia showing diffuse nuclear p53 immunostaining. (p53 IHC staining x400)

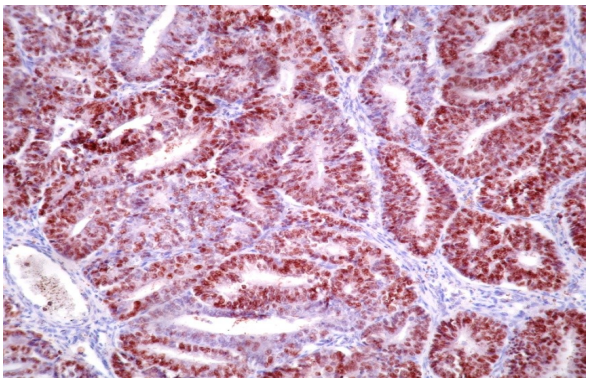

Figure2: Endometrioid carcinoma (grade I) showing diffuse nuclear p53 immunostaining. (P53 IHC staining X200)

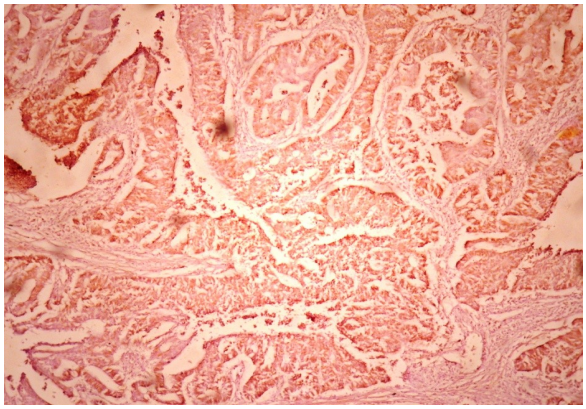

Figure 3: Endometrioid carcinoma (grade II) showing diffuse nuclear p53 immunostaining. (p53 IHC staining x100)

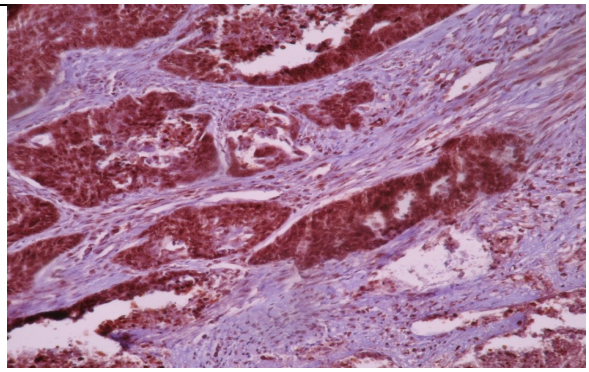

Figure 4: Endometrioid carcinoma (grade III) showing diffuse nuclear p53 immunostaining. (p53 IHC staining x200)

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