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Study of CK7 and HOXB4 Expression in Ovarian Malignancy, Benign and Normal Tissues

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Abstract **Original Research Article**

Ovarian carcinoma (OC) is one of commonest cancers ranks 7th in both incidence and mortality among women worldwide. The study included fifty three samples of ovarian tumors patients, twenty eight of them were malignancy and twenty five were benign. Those patients' samples then compared with twenty three free-cancer tissues samples as control group. This study was carried out in Laboratories of the College of Science/ Department of Biology, Wasit University; during period between 1 November 2017 to 1 November 2018. The study of CK7 and HOXB4 genes expression was evaluated by immunohistochemistry technique. The results showed significant increase of CK7 and HOXB4 expression in ovarian cancer patients when compared with control group and benign patients ($p<0.05$). Moreover, the results showed no significant between benign patients and control group in the expression and intensity of CK7 and HOXB4 ($p>0.05$).In conclusion, the present study confirms that overexpression of CK7 and HOXB4 play necessary roles in carcinogenesis and development of ovarian malignancy.
Keywords: Cancer, benign, ovarian, CK7, HOXB4, gene expression.

INTRODUCTION

Ovarian cancer (OC) is the eighth most common cancer in women worldwide, accounting for 4,4% of cancer in woman ,approximately 295.414 women were diagnosed with this disease in 2018 with about 1.9% mortality rate [1]. The high mortality is primarily due to difficulties in diagnosing early stage disease. The majority of women (75%) are diagnosed at an advanced stage (stage III or IV) [2]. Ovarian cancer is the most lethal gynecologic cancer [3]. It affects women of all ages, but is most commonly diagnosed in those 55 to 64 years of age [4].

Ovarian tumors arise from surface epithelium, sex cord stroma and germ cells. Overall incidence of ovarian neoplasm is surface epithelial (65%), germ cell (15%), sex cor stromal (10%), metastases (5%) as per WHO [5]. Symptoms most predictive of ovarian cancer include persistent abdominal distension, postmenopausal bleeding, appetite loss, and early satiety .These symptoms usually only manifest themselves late in the disease process; hence, 70% of women are diagnosed with advanced stage ovarian cancer [6].Standard treatment involves debulking surgery followed by a combination of taxane and platinum-based therapy. Initially most women respond to platinum-based therapy, but the majority suffers disease recurrence due to drug resistance. It is therefore essential to introduce new therapeutic approaches to improve treatment at diagnosis and/or provide an effective second line treatment [7].

Cytokeratins (CKs), a set of polypeptides of different molecular weights, comprise the main type of intermediate filaments in epithelial cells and provide scaffold structures within cells [8]. More recently, keratins have also been recognized as regulators of other cellular properties and functions, including apico-basal polarization, motility, cell size, and protein synthesis and membrane traffic and signaling. In cancer, keratins are extensively used as diagnostic tumor markers, as epithelial malignancies largely maintain the specific keratin patterns associated with their respective cells of origin [9]. CK7 is the most helpful marker to differentiate primary ovarian carcinoma from metastatic colorectal carcinoma of the ovary. Nearly, 96% of ovarian adenocarcinomas were positive for CK7 in contrast to metastatic colorectal, which showed only 25% positivity. CK7 is useful markers to differentiate primary serous tumors from primary mucinous tumors [10].

HOXB4 gene is a member of the Antp homeobox family and encodes a nuclear protein with a homeobox DNA-binding domain. It is included in a cluster of homeobox B genes located on chromosome 17. The encoded protein functions as a sequence-specific transcription factor that is involved in development. Intracellular or ectopic expression of this protein expands hematopoietic stem and progenitor cells in vivo and in vitro, making it a potential candidate for therapeutic stem cell expansion. [11]. in humans, HOXB4 has been studied extensively in the hematopoietic stem (HS) and progenitor cells in vivo and in vitro [12]. Aberrant expression of HOX genes in epithelial ovarian cancer (EOC) is thought to be a contributor in ovarian tumor progression [13]. HOXB4 has invasion-suppressive effects on ovarian cancer cells, and this effect is partially mediated by CD44 [14]. This study was designed to evaluate the CK7 and HOXB4 expression in ovarian malignancy, benign and normal for diagnostic and confirmation of ovarian cancer.

MATERIALS AND METHODS

Patient's group study

Fifty three tissue samples were collected from ovarian tumor patients, twenty eight of them were diagnosed as OC patients with age ranged between 19 and 81 years, twenty five of theme were recorded as a benign tumor patients with age range between 11 and 70 years were included in this study. All samples of patients were collected from Al- Karama and AL-Zahraa Teaching Hospitals in Wasit Province, and form Private Laboratory (Ibn Al-Bitar) in Thi Qar Province. All patients are diagnosis as malignancy, benign or free cancer patients by physicians and pathologists. Clinical information were obtained involving, clinical examination, and histopathological parameter include type, age, stage and grade .All tissue samples were embedded in paraffin. Twenty three of free cancers ovarian tissues that embedded in paraffin were taken as a control group.

Immunohistochemistry (IHC)

CK7 and HOXB4 antibodies and ABC staining system which was used provided by Abcam Biotechnology. Serial tissue sections were cut 4- 5µm thick and positioned on positive charged slides. The slides were packed at 60-65˚C in oven for overnight .The tissue sections were deparaffinized; then the slides were rehydrated by graded ethanol concentration (100%, 95%, and 70%) and xylene concentration (100%) and distal water. The slides were treated with tris EDTA for 20 minutes, and then washed twice in distal water for 2 minutes. After preparation of tissue sections, incubated in hydrogen peroxide (H2O2) diluted in D.W. for 10 minutes. Each slide was washed in PBS twice for 5 minutes. Sections were incubated for ten minutes in protein blocking serum diluted with PBS. Primary antibody was applied over night at room temperature or overnight at 4° C in humidity chamber. Slides were washed with three changes of PBS for 5 minutes each, and then slides were incubated for 60 minutes with biotinylated secondary antibody and washed with two times of PBS for 5 minutes. Sections were incubated for 10 minutes with streptavidin peroxides, after that washed with three times of PBS for 5 minutes then the section were incubated with DAB stain for 15 min. The slides were washed in PBS, for 3 min for three time. Hematoxylin stain was added on slides for 43 seconds. Immediately, slides rinsed with running tap water for 2 minutes. Dehydrated sections as follows: 1x 95% ethanol for 20 seconds and absolute ethanol at 2 times for 20 seconds and xylene at 1 time for 10 seconds. Immediately 1-2 of DPX solution was added and cover with glass coverslip. Finally, slides were observed by light microscope.

Ethical consent

The study was submitted and approved by the College of Science, University of Wasit in cooperation with ALKarama and AL-Zahraa Teaching Hospitals, Wasit – Iraq.

Statistical analysis

For all statistical analyses, the SPSS system for personal computer was used, and p values of 0.05 or less were regarded as statistically significant. Sensitivity and specificity of the tests (with 95% exact confidence intervals) were determined in studied group. Comparison between groups was carried out using Chi-square test.

Scoring system

Based on the percentage of stained cells and the intensity of nuclear stain. The staining and intensity were scored as follows: The percentage of positive staining (P) were scored as, 1 (1%-25%), 2 (26%-50%), and 3 (51%-100%), and the levels of staining intensity were determined as: 0 for negative staining; 1 for weak staining; 2 for moderate staining; and 3 for strong staining.

RESULTS AND DISCUSSION

Expression of CK7 and HOXB4

CK7 expression and intensity in ovarian cancer and control group

Table (1) referred to the expression of CK7 in ovarian cancer patients in comparison with control group. Expression of CK7 was reported positive in 26(92.1%) of ovarian cancer patients out of 28 cases and the rest 2 (7.1%) cases were showed negative staining , while in control group 6(26.1%) out of 23 cases were showed positive staining for CK7 and the rest 17 (73.9%) cases were showed negative staining. There was highly significant (P< 0.001) difference between patients of ovarian cancer and control group in relation to CK7expression (Figure: 3.1). Intensity assessment of CK7expression in ovarian cancer patients showed that 10 (38.5) cases with score +1, 6 (23.1 %) cases with score +2 and 10(38.5%) cases with score +3.

While in control group, 3 (50%) cases with score1, 1(16.7%) case with score +2 and 2(33.3%) cases with score+3. There were non-significant (P≥0.05) difference between patients of ovarian cancer and control group in relation to intensity of CK7 expression. (Figure: 1).

Table-1: Ck7 expression and intensity in ovarian cancer and control group

Case	Expression		Total	P value	Intensity			Total	P value
	+	-			1	2	3		
	No%	No%			No%	No%	N0%		
Ovarian cancer patients	26 92.9%	2 7.1%	28	P≤0,01	10 38.5%	6 23.1%	10 38.5%	26	P≥0.05
Control group	6 26.1%	17 73.9 %	23		3 50%	1 16.7%	2 33.3%	6	
Total	32	19	51		13	7	12	32	

The results of Cathro and Stoler, Showed that almost all primary ovarian mucinous cancer were positive for CK7 [15]. In addition, Conklin and Gilks, showed that most of the primary ovarian cancer were positive for CK7 [16]. Regarding to immunohistochemical study done by Kriplani and Patel, on ovarian cancer patients, they showed that 96% of ovarian adenocarcinomas were positive for CK7 [10].

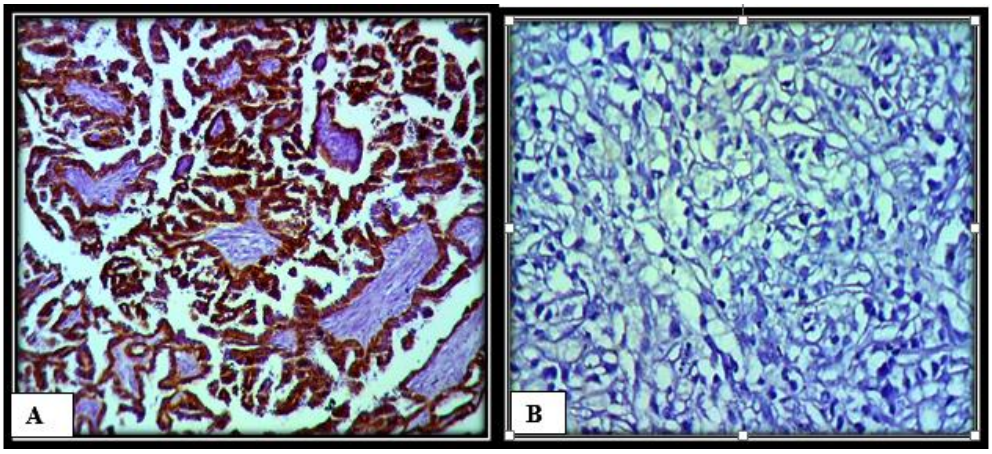

Fig-1: CK7 IHC staining in ovarian cancer patients and control group. (A) Positive staining, (B) Negative (no staining), (40X)

HOXB4 expression and intensity in ovarian cancer and control group

Table (2) showed expression of HOXB4 in ovarian cancer patients in comparison with control group. Expression of HOXB4 was reported positive in 26 (92.9 %) of ovarian cancer patients out of 28 cases and the rest 2 (7.1%) cases were showed negative staining for HOXB4, while in control group 11 (47.8%) out of 23 cases were showed positive staining for HOXB4 and the rest 12 (52.2%) cases were showed negative staining for HOXB4. There was highly significant (p≤0.001) difference between patients of ovarian cancer and control group in relation to HOXB4 expression (Figure: 2).Intensity assessment of HOXB4 expression in ovarian cancer patients showed that 6 (23.6%) cases with score +1, 11 (42.3%) cases with score +2 and 9 (34.6%) cases with score +3. While in control group, 1 (9.1%) case with score +1, 4(36.4%) cases with score+2 and 6(54.5) cases with score+3. There was no significant (p≥0.05) difference between patients of ovarian cancer and control group in relation to intensity of HOXB4 expression .As show in (Figure 2).

Table-2: HOXB4 expression and intensity in ovarian cancer and control group

Case	Expression		Total	P value	Intensity			Total	P value
	-	+			1	2	3		
	No%	No%			No%	No%	No%		
Ovarian cancer patients	26 92.9%	2 7.1%	28	P≤0,01	6 23.6 %	11 42.3%	9 34.6%	26	P≥0.05
Control group	11 47.8%	12 52.2%	23		1 9.1%	4 36.4 %	6 54.5%	11	
Total	37	14	51		7	15	15	37	

Results of Li *et al*. showed that HOXB4 expressions at both mRNA and protein levels were increased in ovarian cancer cells compared with human normal ovarian epithelial cells (HOEC) HOXB4 is just HOX family gene showed significantly higher levels of expression in ovarian cancer cell lines than in normal ovarian tissue (p = 0.029) [17]. Study of Kelly, noted that HOX expression in tumors of a specific tissue is clearly different from the expression observed in the normal tissue. In addition, epigenetic variations can result in the down-regulation or silencing of certain HOX genes which normally act as tumor suppressor's gene. Many studies have demonstrated deregulated HOX gene expression in cancer containing lung, prostate, and breast, colon, bladder and thyroid cancer and also in ovarian cancer [18]. According to study of Hong, *et al*. who use RT-PCR analysis on cDNA derived from 4 ovarian carcinoma cell lines and 4 normal ovarian tissues ,who found that HOXB4 gene was had significantly deference in expression in ovarian cancer cell lines than in the normal ovarian tissues (P<0.029) [19].

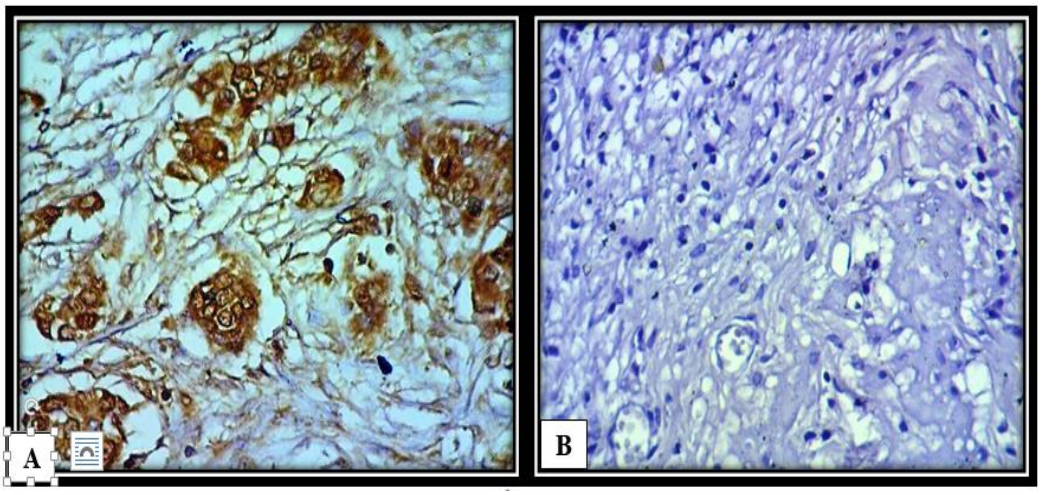

Fig-2: HOXB4 IHC staining in ovarian cancer patients and control group.
(A) Positive staining, (B) Negative (no staining), (40X)

CK7 expression and intensity in ovarian cancer and benign patients

Expression of CK7 was reported positive in 26(92.9%) of ovarian cancer patients out of 28 cases and the rest 2 (7.1%) cases were showed negative staining for CK7, while in benign patients 15 (60%) out of 25 cases were showed positive staining for CK7and the rest 10 (40%) cases were showed negative staining for CK7. There was significant (P≤0.01) difference between ovarian cancer patients and benign group in relation to CK7 expression. Intensity assessment of CK7 expression in ovarian cancer patients showed that 10(38.5%) cases with weak staining, 6(23.1 %) cases with moderate staining, and 10 (38.5%) cases with strong staining. While in benign patients, 5(33.3 %) cases with weak staining, 4(26.7%) cases with moderate staining, and 6 (40%) cases with strong staining. According to above data, there are no significant differences between ovarian cancer and benign patients in relation to intensity of CK7 expression (Table3).

Table-3: Ck7 expression and intensity in ovarian cancer and benign patients

Case	Expression		Total	P value	Intensity			Total	P value
	+	-			1	2	3		
	No%	No%			No%	No%	No%		
Ovarian cancer patients	26 92.9 %	2 7.1%	28	P≤0,01	10 38.5%	6 23.1%	10 38.5%	26	P≥0.05
Benign patients	15 60%	10 40%	25		5 33.3%	4 26.7%	6 40%	15	
Total	41	12	53		15	10	16	41	

The results of Goel *et al*. which involving comparison between cancer and benign patients. They showed significant difference (p<0.001) in expression and intensity of CK7 between benign and cancer patients [20].

HOXB4 expression and intensity in ovarian cancer and benign patients

Expression of HOXB4 was reported positive in 26(92.9%) of ovarian cancer patients out of 28 cases and the rest 2 (7.1%) cases were showed negative staining for HXB4, while in benign patients 13 (52%) out of 25 cases were showed positive staining for HOXB4 and the rest 12 (48%) cases were showed

negative staining for HOXB4. There was significant (p≤0.01) difference between ovarian cancer patients and benign group in relation to HOXB4 expression. Intensity assessment of HOXB4 expression in ovarian cancer patients showed that ,6(23.1%) cases with score +1,11(42.3%) cases with score +2 and 9(34.6%) cases were with scored +3.While in benign patients , 7(53.8%) cases with score +1,3(23.1%) cases with score +2 and 3(23.1%)cases with score +3. There was no significant (P≥0.05) difference between ovarian cancer and benign patients in relation to intensity of HOXB4. As show in (Table 4).

Table-4: HOXB4 expression and intensity in ovarian cancer and benign patients

Case	Expression		Total	P value	Intensity			Total	P value
	+	-			1	2	3		
	No%	No%			No%	No%	No%		
Ovarian cancer patients	26 92.9%	2 7.1%	28	P≤0,01	6 23.1%	11 42.3%	9 34.6%	26	P≥0.05
Benign patients	13 52%	12 48%	25		7 53.8%	3 23.1%	3 23.1%	13	
Total	39	14	53		13	14	12	39	

Our study showed that the HOXB4 expression was significantly increased in ovarian cancer (92%) in comparison with ovarian benign patients (52%). Results of Naora, H. *et al*. demonstrated that one of HOX family gene member showed that significant expression was detected by RT-PCR analysis in serous and endometrioid carcinomas and the expression was also strongly detected in serous cyst adenoma. The current finding suggests, that HOXB4 expression in ovarian cells could be involving in the process leading to normal and benign ovarian tissues transforming to cancerous cells [13].

CK7 expression and intensity in benign patients and control group

Expression of CK7 was reported positive in 15 (60%) of ovarian benign patients out of 25 cases and the rest 10 (40%) cases were showed negative staining for CK7, while in control group 6 (20%) out of 23 cases were showed positive staining for CK7and the rest 17 (80%) cases were showed negative staining for CK7. There was significant (P≤0.01) between ovarian benign patients and control group in relation to CK7 expression. Staining intensity assessment of CK7 expression in ovarian benign patients showed that 5(33%) cases with weak staining , 4(27%) cases with moderate staining and 6(40%) cases with strong staining .While in control group, 3(50 %) cases with weak staining ,1(16.7%) case with moderate staining and 2(33.3%) cases with strong staining . According to above data, there was no significant difference between ovarian benign patients and control group in relation to intensity of CK7 expression (Table5) and (Figure 3).

Table-5: CK7 expression and intensity in benign patients and control group

Case	Expression		Total	P value	Intensity			Total	P value
	+	-			1	2	3		
	No%	No%			No%	No%	No%		
Benign patients	15 60%	10 40%	25	P≤0,01	5 33%	4 27%	6 40%	15	P≥0.05
Control group	6 20%	17 80%	23		3 50%	1 16.7%	2 33.3	6	
Total	21	17	48		8	5	8	21	

Goel, *et al*. cleared that CK7 was showed positive staining in benign and control group. This difference may be due to the effect of CK7 protein on apoptosis in ovarian benign cell [20].

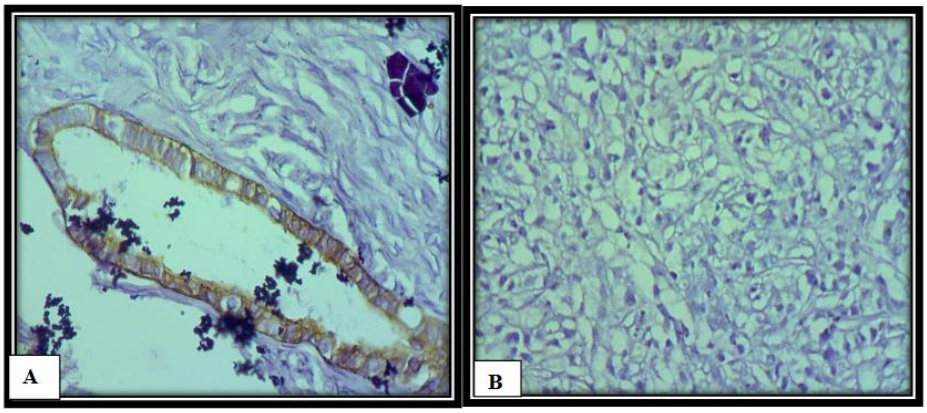

Fig-3: CK7 IHC staining in ovarian benign patients and control group. (A) Positive staining, (B) Negative (no staining) (X**40**)

HOXB4 expression and intensity in benign patients and control group

Expression of HOXB4 was reported positive in 13 (60%) of ovarian benign patients out of 25 cases and the rest 12 (40%) cases were showed negative staining for HOXB4 , while in control group 11 (52%) cases out of 23 cases were showed positive staining for HOXB4 and the rest 12 (48%) cases were showed negative staining for HOXB4. There was no significant (**P≥0.05**) difference between ovarian benign patients and control group in relation to HOXB4 expression. Intensity assessment of HOXB4 expression in ovarian benign patients showed that 7(53.8%) cases with weak staining, 3(23.1%) cases with moderate and strong staining .While in control group, 1(9.1 %) case with weak staining, 4(36.4%) cases with moderate staining, and 6 (54.5%) cases with strong staining. According to above data, there was no significant difference between ovarian benign patients and control group in relation to intensity of HOXB4 expression, as shown in figure (4), (Table6).

Table-6: HOXB4 expression and intensity in benign patients and control group

Case	Expression		Total	P value	Intensity			Total	P value
	+	-			1	2	3		
	No%	No%			No%	No%	No%		
Benign patients	13 60%	12 40%	25	P≥0.05	7 53.8%	3 23.1%	3 23.1%	13	P≥0.05
Control group	11 52%	12 48%	23		19.1%	436.4%	6 54.5%	11	
Total	24	24	48		8	7	9	24	

The results of Li *et al*. showed that HOXB4 expression at both mRNA and protein levels were increased in ovarian tumors cells when compared with human normal ovarian epithelial cells [17].

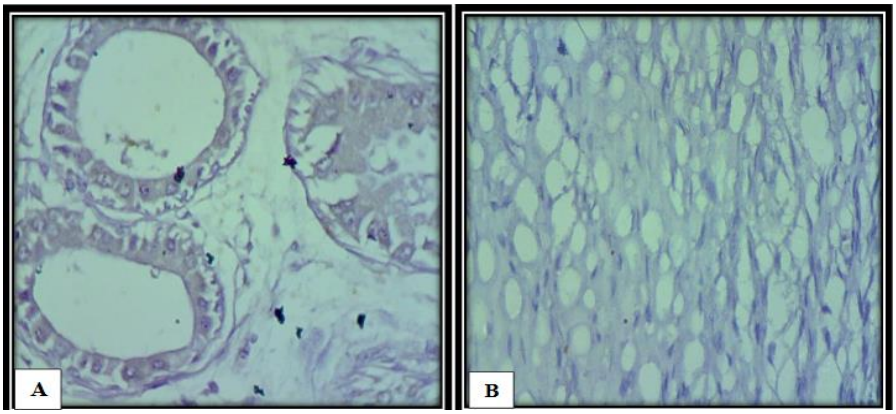

Fig-4: HOXB4 IHC staining in ovarian benign patients and control group. (A) Positive staining, (B) Negative (no staining) (**x40**)

CONCLUSION

Our results concluded that the CK7 and HOXB4 play an important role ovarian carcinogenesis, and represents good tools to diagnosis and follow up of ovarian cancer develop.

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