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HOX-B4 Gene Expression Associated with Stage, Grade, and Invasiveness in Endometrial Carcinoma

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Abstract

Mainly, endometrial carcinoma(EC) was reported as most common malignancies overall. It primarily affects postmenopausal women. Its develops from endometrium and was categorized into two types based on the development mechanism: responsive and non-responsive to estrogen, with dramatically different gene expression profiles, HOXB4 is an important transcription factor that regulates gene expression and plays a key role in the self-renewal and expansion of hematopoietic stem cells. the study's goal was to to evaluate the relation between HOXB4 gene expression and clinic pathological parameters including stage, grade, endometrial invasion, and lymph node involvement. We wanted to characterize the modifications of tumor benign, malignant and normal cases and evaluate the correlations between the HOX-B4 expression and pathological factors including stages, grades, muscles and lymph nodes invasiveness. Principally, HOX-B4 expression value showed up-regulation inHOX-B4 expression that a significant differences (P-value < 0.001) between EC patients and healthy control group. These outcomes also show significance differences (P-value< 0.001) between patient group with endometrial benign tumour and the control, as well as a significant differences (P-value<0.001) between patient and those with benign tumors.

Keywords: Endometrial cancers, HOX-B4, tumors malignant, endometrial benign tumor.

التعبير الجيني لـ *HOX-B4* المرتبط بمرحلة ودرجة وغزو سرطان بطانة الرحم

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قسم علوم الأدلة الجنائية، كلية العلوم، جامعة واسط، واسط، العراق

الخلاصة

يُعد سرطان بطانة الرحم أكثر أنواع السرطانات شيوعًا بشكل عام، ويصيب في المقام الأول النساء بعد انقطاع الطمث. ينشأ هذا السرطان من بطانة الرحم، ويُصنف إلى نوعين بناءً على آلية تطوره: النوع المستجيب للإستروجين والنوع غير المستجيب له، مع اختلاف كبير في أنماط التعبير الجيني. يُعد HOXB4 عامل نسخ مهمًا يُنظم التعبير الجيني، ويلعب دورًا رئيسيًا في التجديد الذاتي وتكاثر الخلايا الجذعية المكونة للدم. هدفت هذه الدراسة إلى تقييم العلاقة بين التعبير الجيني لـ HOXB4 والمعايير السريرية المرضية، بما في ذلك المرحلة، والدرجة، وغزو عضلة الرحم، وانتشار السرطان إلى العقد الليمفاوية. سعينا إلى تحديد التغيرات في حالات الأورام الحميدة والخبيثة والطبيعية، وتقييم الارتباطات بين التعبير عن HOXB4 والعوامل المرضية، بما في ذلك المراحل، والدرجات، وغزو عضلة الرحم، وانتشار السرطان إلى العقد الليمفاوية. أظهرت قيمة التعبير عن جين HOX-B4 ارتفاعًا ملحوظًا في مستوى التعبير عنه، مع وجود فروق دالة إحصائيًا (قيمة $P < 0.001$) بين مرضى سرطان بطانة الرحم ومجموعة التحكم السليمة. كما أظهرت هذه النتائج فروقًا دالة إحصائيًا (قيمة $P < 0.001$) بين مجموعة المرضى المصابين بأورام حميدة في بطانة الرحم ومجموعة التحكم، بالإضافة إلى فروق دالة إحصائيًا (قيمة $P < 0.001$) بين المرضى المصابين بأورام حميدة وأولئك المصابين بأورام حميدة.

1. Introduction

Endometrial cancer(EC) represent sixth mostly prevalent malignant in female, and while it was known to affect postmenopausal women, its numbers among premenopausal women is increasing [1]. Endometrial carcinoma (EC) develops in the inner epithelial lining of the uterus. In 2020, the age-standardized incidence and death rates were 8.7 and 1.8 per 100,000 people, respectively [2]. Endometrial cancer is often classified into two categories, with type

I accounting for the vast majority of instances. Type I comprises endometrial EC, which has a favorable prognosis and is estrogen- dependent. Type II EC includes non-endometrium EC and high-grade EC with a poor prognosis [3]. Traditionally, EC is divided into two major histologist sub type. Endometrial adenocarcinoma accounts in about 70% of recorded cases. This type of tumor usually low-grades and could be detected early. Non-endometrium cancers, on the other hand, are more aggressive and frequently detected at advanced stages. Although its prognosis is generally favorable, it deteriorates significantly when identified at an advance stages, with median of survival period fewer than 12 month [4].

HOXB4gene encodes a nuclear protein with a homeobox DNA-binding domain and belongs to the family. It is part of clusters of homeobox B gene on the chromosome 17. It encodes proteins works as sequencing-specific transcription factors in development. This protein's ectopic or intro cellular expression multiplies hematoma-poietic progenitor and stem cell in-vitro and in-vivo, this makes it a prospective therapeutic stem cells expansion option [5].

2.Materials and Method

2.1.Sample Collection

45 endometrial tissue samples, 15 of which had been previously diagnosed, their ages ranging from 27 to 80 years old, while the remaining 20 cases had been documented as benign tumors, their ages ranging from 23 to 58 years, and 10 samples of no cancer tissues with ages ranging from 31 to 53 years were diagnosed as hormonal imbalance. All patient samples were taken from Wasit Province including both AL-Karama and Al-Zahraa Teaching Hospital, as well as from the Private Laboratory (BioTech) AL-Kut.

2.2.Handling and Immediate Processing

To ensure optimum fixation, tissue samples were immersed immediately after retrieval in 10% buffered formalin. Resect samples were carefully marked to indicate their orientation, particularly for larger specimen. Orientation marker, like as dyes or sutures had been help to maintain the hoist-logical contexts and allowed for more perfect path-logical interpretations.

2.3.Tissues Fixation

It is an essential to ensure preservation of tissue and cellular architectures in order to obtain correct histological evaluation. Fixation processes is critical to stabilizing tissue component, this will prevent deteriorating after sampling. Histological sections are then sectioned using a specialized procedure to create slides tailored to the type and invasiveness of the cancer.

2.4.Molecular researches regards HOXB4 gene role in tumor-endometrial

The following steps had been taken to extract total RNA from tissues sample utilizing the RNA extractions-protocol (GENEzol™ TriRNA Pure Kit, cat# GZX100/D100) as per company instructions: Departmentalization, RNA extraction, purification, and reverse transcription-by real time PCR can be approximated by the equation

$$\Delta\Delta\mathrm{Ct} = \Delta\mathrm{Ct}\,(\mathrm{sample}) - \Delta\mathrm{Ct}\,(\mathrm{control}) \quad (1)$$

where Ct = the number of cycles required for the device to detect the gene, ΔCt = the difference between the target gene and the housekeeping gene, ΔΔCt = the difference between the sample and the control.

2.4.1. PCR working reaction

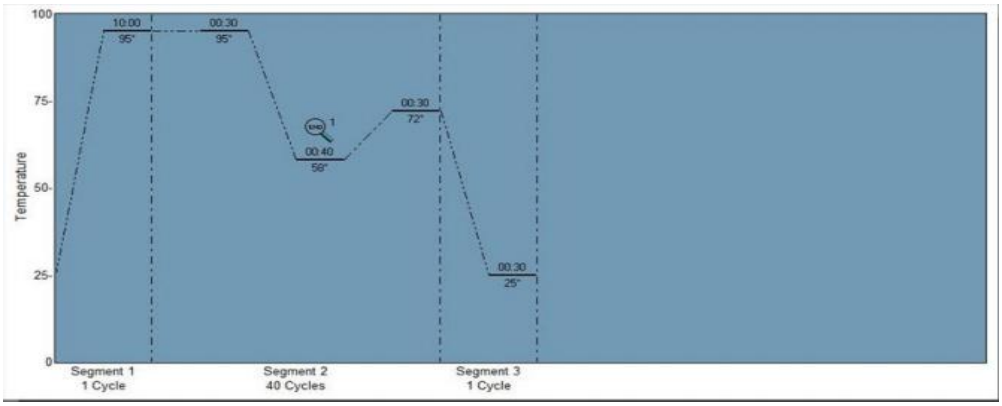

Figure -1 This figure depicts a PCR Programs of HOXB4 gene

2.4.2. Primers dilution

Table 1- Primers sequences of HOXB4 gene

Primer	**Sequence (5'-3')**
HOX11 F	TGCCAAGTTGTACTTACTACGTC
HOX11 R	GTTGGAGGAGTAGGAGTATGTA
GAPDH F	TGCACCACCAACTGCTTAG
GAPDH R	CAGGCAGGGATGATGTTC

2.5. Statistical Analysis

Data were input and analyzed using version 26 of the Statistical Packages for Social Science (SPSS) software. All numerical variable had been symbolized by mean (centrals tendency), standard deviation (SD), whilst categorical variable had been recorded as frequency and percentage. Bar chart and Histogram diagram also were utilized. The mean differences of numerical continuous variables were assessed using the independent samples t-test and the one-way ANOVA test, respectively. Instead of the chi-square test, the Fisher's Exact Test was employed to determine the relationship between two categorical variables (more than 20% of the anticipated cells were fewer than 5). Consider a P-value of 0.05 or less to be significant.

3. Results

3.1. Distribution of endometrium patients according to aging group

The study included 45 endometrial patients with an average age of 46.28±12.25 years. Minimum age represented by 23 year, while the maximum was 80 years old. Current study examines age distributions of cases having endometrial samples, ranging from 20 to 80 years old. Similarly, 23 years old was minimum and 80 the maximum one. Those cases had been divided in-to four groups according to age each with a fifteen-year interval.
first group 20–34 year and last one was 65–80 year. Frequency of patient age groups revealed 9 instances (20%) in age group 1 (20-34), 16 cases (35.4%) in the age group 2 (35-48), 17

case (37.7%) in the age group 3 (50-64), and 3 case (6.6%) in the age group 4 (65-80). Present findings are also presented in figure (2) with age interval and table (2).

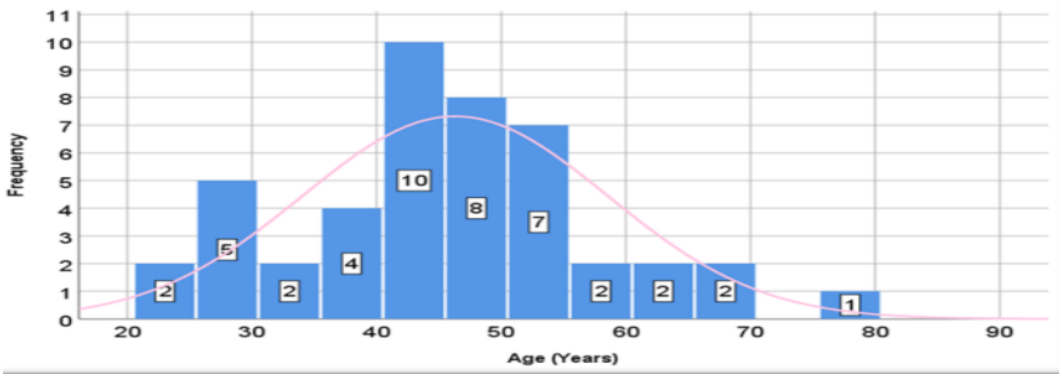

Figure- 2 This figure depicts a patients age distribution with age intervals.

Table 2- Distribution of the 45 endometrium patients according to aging group

Variable	Minimum Maximum	Mean	Standard Deviation
Age (Years)	23-80	46.29	12.26
	Age groups	Frequency	Percentage
	20-34	9	20%
	35-49	16	35.4%
	50-64	17	37.7%
	65-80	3	6.6%

3.2. Distribution of patients age according to endometrium histo-pathology diagnosis.

Present study displays the distribution 45 sample from endometrial patient according on their diagnosis.As a control group, 15 (33.6%) patients had EC cancer (malignant tumor), 20 (44.2%) patient had endometrial benign tumor, and 10(22.2%) patient had no pathological alteration in the endometrium but complained of hormonal imbalance. This study discovered that the mean age and standard deviation were similar in endometrial patient samples.There were 55.60±10.60 patients with endometrial cancer tumors, 40.70±12.40 with endometrial benign tumors, and 43.50±5.96 with endometrium control ,Age distribution based on endometrial histopathology diagnosis among 45 individuals.

Table 3- Distribution of age according to endometrium histopathology diagnosis among 45 patients.

Histopathological Diagnosis	Frequency	Percentage	Mean age	S.D	P-value
Endometrial cancer tumors	15	33.6%	55.60	12.40	0.001
Endometrial benign tumors	20	44.2%	40.70	10.60	
Endometrium control (Hormonal imbalance)	10	22.2%	43.50	5.96	
Total	45	100%			

3.3. Distribution of malignant endometrium patient according to histo-pathological types

Four histologist type were identified in endometrial a cancer patient. 11 patients (72.3%) had endometrium adenocarcinoma, 1 (6.6%) had serous endometrial adenocarcinoma,2 (12.3%) had Villoglandular Adenocarcinoma, and 1 (6.6%) had Malignant Mixed Mullerian Tumor(Table 4).The distribution of malignant endometrial patients on histologist type.

Table 4- Malignant endometrial patients distribution acording to histo-pathological types

Histotype	Frequencies	Percentages
Endometroid Adenocarcinoma	11	72.3%
Serous Endometrial Adenocarcinoma	1	6.6%
Villoglandular Adenocarcinoma	2	12.3%
Malignant Mixed Mullerian Tumor	1	6.6%

3.4. Histotype of malignant endometrial tumor and age groups association.

present study included individuals with malignancy endometrial tumors aged 27 to 80 year. Minimum age has been recorded 27 year, and maximum was 80. Malignant endometrial tumor patient had been divided in to four group, with a fifteen year interval. First group (20-34) year, and latest was (65-80)year. Anestimation of malignancy endometrial age group revealed that 1 case (100%) with age group1(20-34) of them were Villoglandular Adenocarcinoma, 2 cases (100%) in age group2 (35-49) with Endometrial Adenocarcinoma, 8 cases (88.9%) with Endometrial Adenocarcinoma and1cases (11.1%) with Villoglandular Adenocarcinoma in age group 3 (50-64) and 1 cases (33.3%)with each of Endometrial Adenocarcinoma, Serous Endometrial Adenocarcinoma and Malignant Mixed Mullerian Tumor The relationship between histotype and age groups in patients with malignant endometrial tumour.

Table 5- Association between histotype and age group of malignant endometrial tumor patient

Age (year)	Histotype				P-value
	Endometroid	Serous Endometrial Adenocarcinoma	Villoglandular Adenocarcinoma	Malignant Mixed Mullerian Tumor	
20-34	0(0%)	0(0%)	1(100%)	0(0%)	0.110
35-49	2(100%)	0(0%)	0(0%)	0(0%)	
50-64	8(88.9%)	0(0%)	1(11.1%)	0(0%)	
65-80	1(33.3%)	1(33.3%)	0(0%)	1(33.3%)	

3.5. Frequencies of distribution of endometrial cancer patient according to grading

Present study included 15 endometrium cancer patient had been distributed into three grade, grade I represented by 12 (80.2%) of patient, while grade-ii recorded 2 (13.3%) of

patient, grade-iii was indicated in 1(6.5%) of patient (Table6). Frequency distribution of the endometrial cancer patient according to grades.

Table 6- Frequency distributions of endometrial cancer patient acording to grades

Grade	Frequency	Percentage
G1	12	80.2%
G2	2	13.3%
G3	1	6.5%
Total	15	100%

3.6. Frequency distributions of endometrial cancer patient according to stages.

In present study, patient were distributed into three stage, first one indicated 14(93.4%) of patient, stage-ii found in 1(6.6%) patient, while stage-iii recorded in 0(0%) of patient, table (7):

Table 7- Frequency distributions of patient according to stages.

Stages	Frequencies	Percentages
I	14	93.4%
II	1	6.6%
III	0	0%
Total	15	100%

3.7. Frequencies distribution of muscle inventiveness among malignant endometrial cancer patients

Endometrium cancer is either invasive or non-invasive muscles. In this study, it was found that 3(20%) of patients suffering from muscles inventiveness endometrium cancer, and other 12 (80%) of them presented with non-invasive Figure (3). Frequency distribution of muscle inventiveness among 15 patients diagnosed with malignant endometrial cancer.

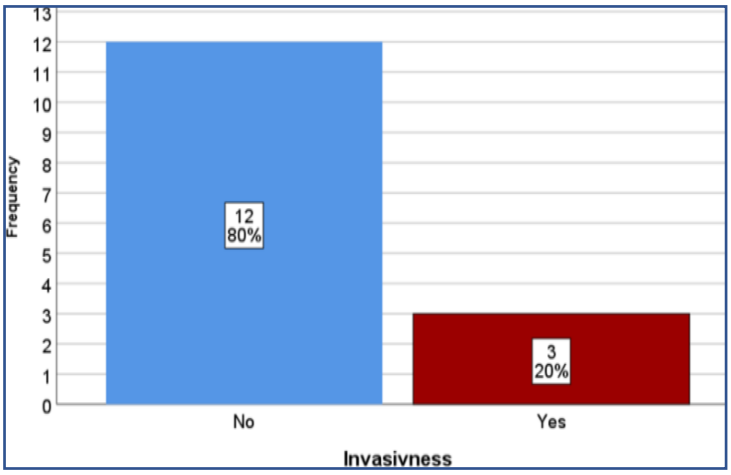

Figure- 3 This figure depicts a Frequency distributions of malignant endometrial cancer patients

3.8. Frequencies distribution of lymph nodes involvements-among malignant endometrial cancer patients

Endometrium cancers either considered as non-invasive or invasive lymph nodes. In current study 13 patients were with invasive lymph nodes Table (8):

Table 8- Frequencies distribution of lymph nodes involvement among patients

Variables	Yes Numbers	No Numbers
Lymph nodes involvement	**1**3	2

3.9. Benign endometrium patient distribution according to histo-pathological types

present study depicts the distributions of 20 patient diagnosed with a benign endometrium tumors. Two types of histological had been identified.12(60.0%) patient had leiomyoma, while 8 (40.0%) had polyps (Table 9).

Table 9- Benign endometrium patient distribution acording to histo-pathological types

Histotypes	Frequencies	Percentage
Endometrial polyp	**8**	**40%**
Leiomyoma endometrium	**12**	**60%**

3.10. Histotype and age groups association among benign endometrial tumors

Current study included individualistically aged 20 to 64 year. Minimum age had been recorded 20, while maximum was 64 year. Patient had been distributed into three group (1-3), with an interval of fifteen-year. First one (20–34) year and last was50–64 year. An estimation of a benign age group revealed that there was no case with polyp, on the other hand 7(100%) cases with leiomyoma with age group 1(20-34), 4(57.2%) cases with polyp while 3(41.9%) cases with leiomyoma with age group 2(35-49) and 4(66.5%) cases with polyp while 2(32.3%) cases with leiomyoma with age group 3(50-64) Table(10):

Table 10- Association between age group and histotype of the benign endometrial tumor.

Age categories (year)	Histotypes		P-value
	Polyp-endometrial	Leiomyoma endometrium	
20-34	0(0%)	7(100%)	0.028
35-49	4(57.2%)	3(41.9%)	
50-64	4(66.5%)	2(32.3%)	

3.11. Expression of HOXB4 in endometrium

3.11.1: Expression of HOXB4 gene in patients

Depicted HOXB4 expression in patients with endometrial cancer and benign tumors vs the healthy group. Its mean of expression was(2.51±0.23) in 15 instances of endometrial cancer out of all cases, (1.21±0.20) in 20 case of endometrial benign out of 45 patients, and(1.08±0.54) in10 cases of endometrial control out of 45 patients (Figure 4).

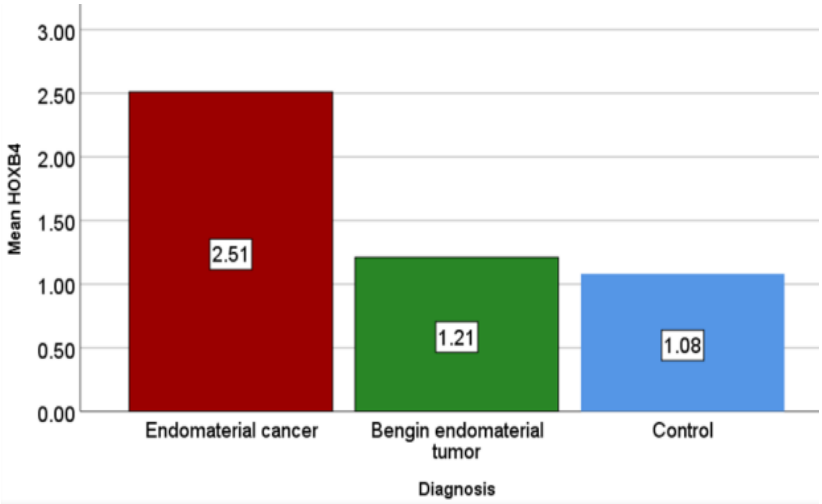

Figure-4 This figure depicts a Mean of HOXB4 gene expression in patient

3.11.2. Mean differences in HOXB4gene expression between control and endometrial cancer tumor patients

According to table (11), mean of HOXB4 expression in endometrial cancer patient was compared to the control group. Endometrial cancer patients had a mean HOXB4 expression of 2.52±0.22 with a standard deviation of 0.22, while endometrial control patients had a mean expression of 1.07±0. 0.55 with a standard deviation of 0.55 (refer to Table 3.24).

Table11- The average differences in gene expresion between endometrial cancer and control.

Groups	Mean	Standarddeviation	Pvalue
Endometrial cancer tumor	2.52	0.22	<0.001
Control	1.07	0.55	<0.001

3.11.3. Mean differences in HOXB4 gene expression between endo-metrial benign tumor and control patient.

Table (12) indicates the mean expression *HOXB4* of endometrial benign patients compared to the control group. The mean expression HOXB4(1.20±0.21) was recorded with a standard deviation (0.20) in endometrial benign patients, and the mean expression HOXB4 (1.06±0.53) was recorded with a Standard deviation (0.54) in endometrial control patients group control patients.

Table 12- Mean differences in HOXB4gene expression between endometrial benign tumor and control patient.

Group	Mean	Standard deviations	Pvalue
Endometrial benign tumors	1.20	0.21	<0.001
Control	1.06	0.53	<0.001

3.11.4. Mean differences in HOXB4 gene expression between endometrial cancer and benign tumors patient.

According to table(13) mean of HOXB4 expression was showed in both endometrial cancer patient compared to endometrial benign tumors patient. Endometrial cancer patients had a mean expression of 2.52±0.24 with a standard deviation of 0.24, while endometrial benign patients had a mean expression of 1.20±0.21 with a standard deviation of 0.21 (see Table). (3.26) The average expression of the HOXB4 gene varies between patients with endometrial cancer and with benign tumors.

Table 13- Differences in the mean expression of the HOXB4 gene between patients with endometrial cancer and benign tumors.

Groups	Mean	Standard deviations	Pvalue
Endometrial cancer tumor	2.52	0.24	<0.001
Endometrial benign tumors	1.20	0.21	< 0.001

4. Discussion

Current study found that age group3 (50-64)years recorded the highest (37.8%) incidence rate in endometrial patients, group 2 (35-49) years was 35.6%. This result indicated that endometrial uterus diseases occur more frequently in older women than 40 years old, and that endometrial uterus diseases are generally not considered a significant risk under the age of 40. This could be related to discontinuous ovulation during the perimenopausal phase, which causes a reduction in progesterone levels because there is no corpus luteum present. The ovaries continue to release estrogen, allowing the endometrium to proliferate; the thicker endometrium grows fast, loses its blood supply, suffers localized necrosis, and begins to shed. The shedding is uneven, and the bleeding is protracted and heavy. Chronic endogenous estrogen stimulation of the endometrium without adequate progesterone levels can result in endometrial hyperplasia and malignancies[1, 6]. This conclusion is consistent with the findings of [7], who reported that diagnosis age were 6% (40) year, 6%(41-50) year, 25%(51-60) year, 42%(61-70) year, and 21% (70) year. Furthermore, [8], found that (44.9 + 7.5) years old was the mean age at diagnosis, these results were so younger than postmenopausal groups (62.3 + 6.5) year.

These findings revealed a substantial age difference(P-value=0.001) across three diagnosis group. Endometrial cancer patients had a greater average age (55.60±12.40) than those with benign illnesses (40.70±10.60) or control group circumstances (43.50±5.96). The study indicated that patients with endometrial cancer had an average age of 61.9 ±11.4 years, which is consistent with finding [9]. the maximum age of the patients was 78 years and the minimum age was 35 years and the most common age group of patients was 56-65 years and explain the age group of (56-65) years was the highest (36.1%), followed by the age group of (66-75) years was (26.2%), with a mean age group of 61.92 ±11.47 (35-78) years. Also, this result is agreed with[10], indicated that aged above 50 year old were more prone to EC. This could be owing to an high estrogen level induced by a variety of factors that continuously drive the development of endometrial tissue, long term estrogen exposuring in the endometrium, and lacking of the progesterone antagonism[11, 12]. Furthermore,[13] reported that mean standard deviation with endometrial benign tumors patients (45.0 ±10.37) and reported that 972 patients with a mean age of(45.77 ± 10.70) ranged from 25 to85 year, 52 (5.3%) of which had suffered from endometrial cancer. A significant differences were reported among groups (malignant and benign) regarding age (p<0.001).

According to the findings, Endometrial Adenocarcinoma is the most common pathological kind among individuals with malignant endometrial malignancies. It was diagnosed in 72.3% of patients, followed by Villoglandular Adenocarcinoma in 12.3% of patients. Only one of the 15 patients (6.6%) was diagnosed with serous endometrial adenocarcinoma and malignant mixed mullerian tumour. Endometrial adenocarcinoma (EEC) are the most common sub-type, accounting for around 80% of cases. Compared to other kinds, EEC are clinically aggressive. According to [14], Serous Endometrial Adenocarcinoma accounts for around 10% of all endometrial cancer diagnosis and up to 39% of endometrial cancer fatalities.Furthermore, [15] identified histology sub-type as serous

(5.6%), mixed type (1.7%), endometrial(87.2%), clear cell(1.7%) and adenocarcinoma(3.9%). This could be due to endometrioid, which is assumed to be hormone-linked and appears to be caused by exposure to high levels of estrogens, whereas other types are estrogen-independent and appear to be unrelated to hormonal exposure (Setiawan et al., 2013). This shows no significant difference between age groups and the histo type of cancer (P-value=0.110). All patients (100%) at age less than 35 were Villoglandular Adenocarcinoma. only two cases with age groups (35-49) years were diagnosed as Endometrial Adenocarcinoma. Result [16], found that diagnosed cases aged was statistically different in histology category (*p*=0:02)with endometrial types tumor presenting on average a younger age (mean =68:5) year than other sub type. In addition, [17], showed that the mean age of patients was 55 years (range 45-74) for group 1 and was 56ys (range 43-75) for group2. Also,[17] explained that Mean age of the patients was 56 years (25–77 years), Endometrioid carcinoma was the commonest histologist type (136 patients, 88%). Other hematological cell types were clear cell carcinoma (14)9%patients, papillary serous carcinoma (3)1.9%patients, and adenosquamous carcinoma (2)1.2% patients.

Outcomes of endometrial cancer among patient in present study was revealed that showed that major proportion was recorded in grade I (80.2%) of patient, followed by grade-ii (13.3%) and (6.5%) in grade-iii. It was concluded that in grade I proportion was higher from both grade-ii and III, This could be because the patients were diagnosed at an early stage with irregular uterine hemorrhage and had surgery soon after. This finding is consistent with result [18], which revealed that the distribution of tumor grade in endometrial cancer was as follows: grade I, 15 (33.33%); grade II, 23 (51.11%); and grade III, 7 (15.56%).Furthermore, [17], discovered that preoperative abnormal tissue grading led in about (73.3%) of patients being grade 1, 20% being grade 2, and 6.5% being grade 3 for both groups. The postoperative results revealed some changes, with approximately (60%) of patients being grade 1, approximately (33.3%) being grade 2, and approximately (6.5%) being grade 3. In group 2, approximately (66.7%) of patients were grade 1, approximately (20%) of patients were grade 2, and approximately (13.3%) of patients were grade 3.

The results of this figure (3) showed that proportion of absent muscle invasion in endometrial cancer patients is higher than that of existent muscle invasion. In this study, inventiveness was observed in approximately 80% of patients, while invasion muscle was present in approximately 20% of those diagnosed with endometrial cancer[18]. Furthermore, [19], explains that non-muscular invasion was 101 (98.06%) of 103 cases with premenopausal EC and 101 (89.38%) of 113 cases with postmenopausal EC, whereas muscular invasion was 2 (1.94%) of 103 cases and 12 (10.62%) of 113 cases with postmenopausal EC, with P(value= 0.079) in both cases. Moreover, [20] explain that in (EC) depth of endometrial invasion none or <50% was 50 (65.8%) cases and in endometrial invasion ≥ 50% was 26 (34.2%) cases.

revealed that endometrial cancer patients have a higher proportion of lymph node invasion compared to those who have no lymph node invasion. The results explain that none lymphatic metastasis was 102 (99.035%) of 103 cases with premenopausal EC and 101 (89.38%) of 113 cases with postmenopausal EC, whereas muscular invasion was 1 (0.97%) of 103 cases and 12 (10.62%) of 113 cases with postmenopausal EC, with P (value = 0.003) in both cases. Furthermore, [21] state that in (EC), lymph node invasion was evident in 26 (34.2%) patients but absent in 50 (65.8%) cases. However, [18] reported that The incidence of lymph node metastasis in high-grade disease was 20% (13 out of 63 patients) and 14% (13 out of 92 patients) in low-grade disease.

This study found that of the 20 patients diagnosed with a benign tumor in the endometrium, 12 (60.0%) were leiomyoma and the remaining 8 (40.0%) were polyps, indicating that leiomyoma is the most prevalent. This finding was consistent with studies [22] which revealed that endometrial polyps were the least commonly identified pathology in the

community. Furthermore, [23] found that benign endometrial polyps occurred in 2 (01.20%) of 7 (04.21%) patients. Also, [24] reveal that leiomyoma is the most common, accounting for 25 cases, while polyps are found in 8 cases of benign individuals.

This study found a significant correlation between histo type of benign tumor patients and their age group (P-value=0.028). All patient aged 20 to 34 years were diagnosed with leiomyoma (70%), while patients aged 50 and up had a polyp (46.5%). The results of [25] show that the benign endometrial polyp 42 (47.8%) had been grouping into 5 age group(1-5) with an interval of 10 year and showed that there were 15 (60%) cases with age group (30-40), 23 (47%) cases with age group (41-50), 4 (36.4%) cases with age group (51-60), while 0 (0.0%) cases with age group (61-70) and (>70) with (P-value<0.0001).

It was also shown that endometrial polyps were the most frequent pathology in all age groups under 60. Furthermore, [26] discovered that the bulk of the instances (56.50%) were seen in the age category of (41-50) years, with the remaining cases (84.29%) seen in the age group of (31-50) years. This may be benign endometriosis that primarily affects postmenopausal women. However, [27] disagreed with the findings and reported just 4% of the women in the postmenopausal age range. Moreover, found that the occurrence of uterine fibroid differs between 5% and 65% based on age, ethnicity, geographical area and quality of imaging technique[28].

These findings revealed the expression of mean HOXB4 levels in three groups of endometrial patients (cancer, benign, and control). Among the three categories, patients with endometrial cancer had the highest mean (2.51±0.23), followed by endometrial benign (1.21±0.20) and patients with endometrial control (1.08±0.54). Y. Xiong et al. (2016)[29] found that the expression of HOXB4 in ectopic and ectopic tissue of endometriosis patient (n=15) compared to healthy females (n=15), asignificant decrease in HOXB4 expression level of in ectopic tissues compared to the ectopic endometrium P=0.0083, and altered expression of specific HOX genes in ectopic and eutopic endometrial tissues compared to the normal endometrium. Furthermore, Philippidou and Dasen (2015)[30] found significant differences in the expression of 3'HOX gene members in the endometrium of the study groups, with the HOXB cluster changing by more than 400-fold. We predicted that these paralogous HOX gene would be under expressed in uterine and endometriosis implant dueto the chromosomal locations.

These data reveal that HOXB4 expression levels was significantly different (P-value< 0.001) between the control group and patients with endometrial cancer. Cancer showed a greater mean (2.52±0.22) than the control group (1.07±0.55). Ludwin, Artur,et al.(2020)[31] hypothesize that lower HOXB4 expression may promote endometrial cancer cell invasiveness by promoting epithelial-to-mesenchymal transition (EMT). Furthermore, Jahromi et al. (2024) [32] found a significant decrease in the eutopic endometrial group compared to the control group (P=0.0007). However, 200 patients with endometriosis had lower HOXB4 levels in their proliferate phase ectopic and ectopic endometrial tissues. Among these tissues, deep infiltrating lesions showed the lowest amounts of HOXB4.

The mean HOXB4 expression value showed a significantly different (P-value<0.001) among cases with benign tumors and control patients. Patients with endometrial benign tumors showed a higher mean (1.20±0.21) compared to the control group (1.06±0.53. Hameed, Z. A., & Mahood, A. K. S. (2024)[33] discovered signification decrease in HOXB4 expression levels in ectopic tissue compared to eutopic endometriumP=0.0083, as well as altered expression of HOX gene in eutopic and ectopic endometrial tissue compare with normal endometrium,additionally, HOXB4 expression in eutopic and ectopic tissue of endometriosis patient(n=15) when compare to healthy female(n=15).Also, AlKusayer *et al*. (2017) suggested that impaired ectopic capacity or ectopic endometrial tissues were related to up-regulation of this gene during proliferative phases, this may related to that it plays arole in

endometriosis pathogenesis. So, further *HOXB4* down regulation could enhance ectopic implant invasivenes.

The mean HOXB4 expression in endometrial cancer patients (2.52±0.24) was substantially greater (P-value <0.001) compared to benign endometrial tumor patients (1.20±0.21). AlKusayer (2018) [34] discovered that HOXB4 expression was considerably lower in DIE lesions than in matching eutopic endometrium and endometrium/deep infiltrating endometriosis (DIE) lesions. After normalizing HOXB4 mRNA levels to endometrial epithelial cell content,HOXB4 mRNA levels were significantly lower in the DIE groups than in EC and endometrial (Eoma). HOXB4 expression is reduced in DIE but not in endometrial endometriosis and may be dys regulated in ectopic implants. In the normal eutopic endometrium, HOXB4 expression in endometrial glandular epithelial cells is higher during the proliferative phase of the menstrual cycle than in the secretory phase. This is the first study to use RTPCR to detect HOXB4 expression in endometrial benign tumor tissues and compare the mean differences in expression of HOXB4 genes between benign tumor and endometrial cancer patient.

5.Conclusions

In this study, we endeavored to understand the differential gene expression patterns of *HOXB4* across varying states of endometrial tissue health, namely, endometrial cancer (EC), benign tumors, and normal controls. Our results have provided compelling insights into the potential roles these gene play in colorectal tumorigenesis and disease progression.

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