PTEN gene mutation in endometrial carcinoma correlation with age and grade

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Abstract :-

The present study has been conducted to evaluate the effect of PTEN gene mutation in endometroid endometrial carcinoma through estimate the correlation PTEN expression with some clinico pathological feature (age and grading) of this cancer. Collect Forty three samples of endometroid endometrial carcinoma and twenty non-malignant samples .all samples were formalin fixed and paraffin embedded tissue collected from january 2008 to 2014 from Iraqi histopathological laboratories , after extract the total RNA the cDNA synthesis and amplification by qRT_PCR then calculate the result via relative (Livak) method. The statistical analyses reveal that no correlation between pten expression and advancing age.on other hand the study establish a positive correlation between PTEN expression and mean grad of tumor.

Keyword : gene , endomaterial carcinoma

Natural history Classification QH 426-470

Introduction

Endometrial cancer is the fourth most common cancer in American United states Women and the most frequently diagnosed gynecologic cancer [1] . In Iraq, Iraq cancer registry resulting between 2004-2008 reveal this cancer was not included within the ten commonest cancers in Iraq [2]. Endometroid endometrial carcinoma actually diagnosis within premenopausal or postmenopausal age period between 50 to 65years with average 61 years [3]. Several risk factor play important role in enhancement and develop this carcinoma involved unopposed estrogen therapy, estrogen –producing tumors, tomexifen treatment, obesity, diabetes mellitus, null parity and infertility [4] .in other hand , combined contraceptive drug and ciggarate smoking considered as protective factors for developing this cancer [4] .WHO classified endometrial cancer according to histological cell pattern to endometroid endometrial carcinoma (most commonly categories account for 75%to 80% of cases [5] . Which are associated with estrogen related and arise from endometrial hyperplastic precursor, the other categories like Mucinous Carcinoma, Serous Carcinoma, Clear Cell Carcinoma, Squamous Cell Carcinoma Undifferentiated Carcinoma, were characterized by association with advance age, atrophic endometrial pattern precursor and less diagnostic account [5]. According to pathogenesis endometrial carcinoma classified to two type, Each one have distinct clincopathological features, pathogenic pathway and gene molecular alteration profile , type 1 endometrial carcinoma characterizes an estrogen-related carcinoma, frequently arises in the setting of endometrial hyperplasia with picture of endometroid histology ,low grade, and tends

to be biologically indolent, molecular PTEN, PIK3CA, KRAS, CTNNB1 (β-catenin) genes and microsatellite instability (MSI), The type 2 carcinoma are not estrogen-driven and have higher grade, a poorer prognosis it consist various histological pattern , particularly serous carcinoma and clear-cell carcinomas, arise from atrophic endometrial precursor and associated with p53 gene mutation [6] . The most common gene mutation in endometroid endometrial carcinoma is PTEN gene [7] . This gene located on chromosome 10q23, is contain 9 exons bridging (128336 base pairs) of genomic DNA This gene encodes protein consist of a 403 amino acid coding from 9 exons, PTEN protein involve several domains have an important role in regulate its activity[7]. this protein also called (phosphatase) can be modifies other proteins changes, including, mutations of and fats (lipids) by removing phosphate groups, this phosphatase enzyme enter in several signal transduction pathways such as (phosphoinositide pathway), MAPK(mitogen-activated protein kinase pathway) and FAK (focal adhesion kinase pathway) lead to stop cells division, start apoptosis, and inhibiting cell spreading [7] . When PTEN function loss the phosphatidylinositol 3, 4, 5-trisphosphate and protein kinase B accumulate with higher levels lead to prevents apoptosis, enhancing the cell cycle continuation then provide uncontrolled cell proliferation and division, another result of PTEN loss function is a defect in cell adhesion lead to improve cell spreading and metastasis because PTEN have an important role in focal adhesion of cells [7] .

Materials and methods:-

Sampling of cases:

This is a case control study performed in department of pathology and forensic medicine medical college of Koufa University in period from first of November 2012 to first of November 2014 using 43 samples taken from patients having endometroid endometrial carcinoma. All samples were formalin-fixed and paraffin-embedded. These either obtaining from curetting materials or from hysterectomy specimen's with an age range of 38 – 70 years. Median age was 59 years. This paraffin- embedded and formalin-fixed samples making by laboratories in Baghdad, Najaf, Diwanyia in period from 2008-2014.also other 20 endometrial samples taken from hysterectomy specimens which are identified as non-neoplastic conditions were used as control groups. Sections will be taken from these specimens for Staining by haematoxylin and eosin staining method to confirm the diagnosis. Then PTEN expression analysis by using qRT-PCR technique, several events doing in this study to extract whole gene from Paraffin embedded samples blocks by modification in period of proteinase k exposure to sample from 3 hours to 3 days to dissociate the cross -linkage between DNA and Histone due to effect of formaldehyde during fixation processing , all our events to separate this total PTEN DNA completely without fragmentations were fail, the most probably reason for this failure due to very long length of this DNA genome ,(128336 base pairs) of genomic DNA)[8] ,instead total DNA extraction , total RNA extraction be done and used as a template to synthesize cDNA then amplification by RT-PCR .

Pten and GAPDH gene Primers and probes

The Pten and GAPDH gene Primers and probes were designed by using NCBI- Gene Bank data base and Primer 3 design online, Appendix (A) these primers were provided by (Bioneer Company, Korea) as following table.

Gene		Sequence
PTEN primer	F	ACCAGTGGCACTGTTGTTTC
	R	TTAGCTGGCAGACCACAAAC
PTEN probe	FAM-	TGTTCAGTGGCGGAACTTGCA-TAMRA
GAPDH	F	TTAAAAGCAGCCCTGGTGAC
	R	TTAAAAGCAGCCCTGGTGAC
GAPDH probe	FAM-A	CCAGCCGAGCCACATCGCTC-TAMRA

RNA isolation and q PCR assay.

Total RNA were extracted from cancer tissue samples by using (TRIzol® reagent kit. Bioneer. Korea) and done according to company instructions. The extracted total RNA was assessed and measurement by Nanodrop spectrophotometer (THERMO. USA), There are two quality controls were performed on extracted RNA. First one is to determine the quantity of RNA (ng/µL), the second is the purity of RNA by reading the absorbance in spectrophotometer at 260 nm and 280 nm in same Nanodrop machine. Then following by treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme kit) and done according to method described by Promega company, USA instructions. DNase-I treated RNA samples were also used in cDNA synthesis step for Pten and GAPDH gene by using M-MLV Reverse Transcriptase kit and done according to company instructions. The quantitative Real-Time PCR used in quantification of Pten gene expression analysis that normalized by housekeeping gene (GAPDH) in cancer and normal adjacent tissue samples by using Real-Time PCR technique. The data results of q RT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) Livak method, In this method, one of the experimental samples is the calibrator such as (Control samples) each of the normalized target values (CT values) is divided by the calibrator normalized target value to generate the relative expression levels. After that, the ΔCT Method with a Reference Gene was used as following equations:

$\Delta\Delta CT = \Delta CT$ (test) – ΔCT (calibrator), Fold change = $2^{-\Delta\Delta CT}$ [16].

Statistical analysis

Data were summarized, presented and analyzed using two software programs. These were the statistical package for social sciences (SPSS) version 16 and Microsoft Office Excel 2007. Numeric (scale) variables were presented as mean, standard error (SE), median and range, while nominal (discrete or categorical) variables were presented as number and percentage. Mann Whitney U test was used to compare variable median between two independent groups, while Kruskal Wallis H test was used to compare variable median among more than two groups. When two numeric variables were to be correlated, Pearson Correlation coefficient test was used, while correlations between two nominal variables were conducted using Kindal Tau-B test. Spearman Rank correlation coefficient test was used to correlate one numeric variable with another nominal variable. P-value of less than or equal to 0.05 was considered significant. In the present study PTEN gene

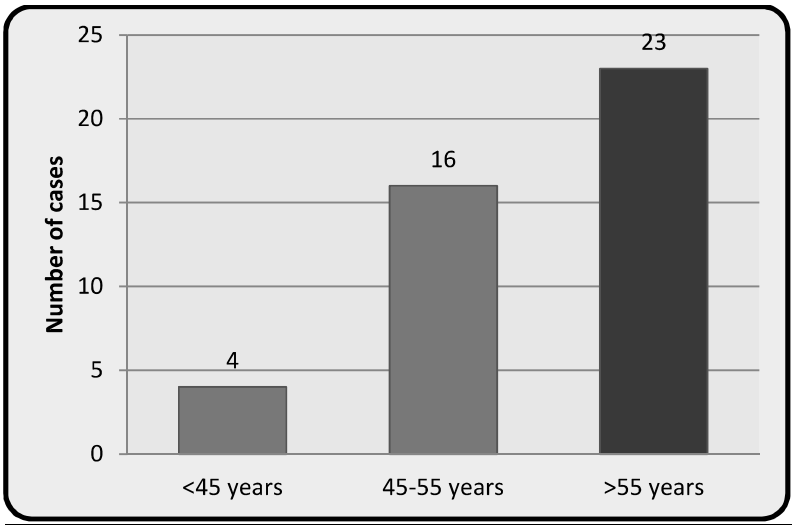
fold change was a numeric variable. This gap among mean, median and mode relatively high. For this reason it was, PTEN gene fold change, treated as a not normally variable has extreme values rendering the distributed variable. That's to say, median is more representative than mean value. .

Result

PTEN gene expression was measure using number of fold change. The description and analysis of incoming results will use this fold change as a numeric variable.

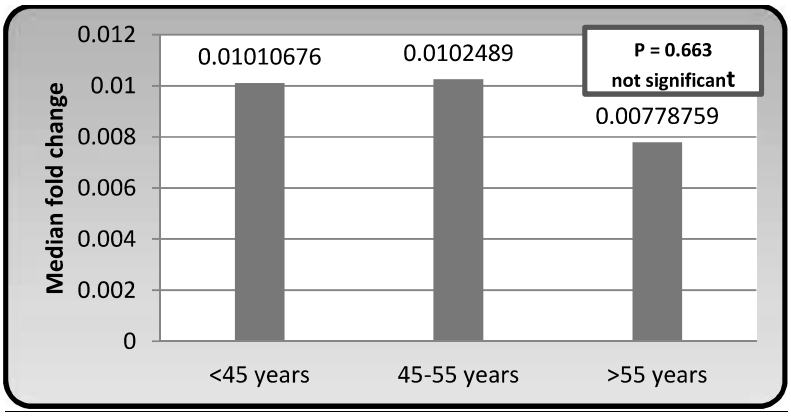
Mean age of women enrolled in the present study

The Mean age of women included in our study was (55.35±1.23) years, with an age range of (38 – 70) years. Median age was (59) years. When patients were classified into three age groups; <45 years old, 45-55 years old and >55 years old, the number of cases was 4, 16 and 23 respectively. It was obvious that majority of cases were more than 55 years old. The figure below illustrate it.



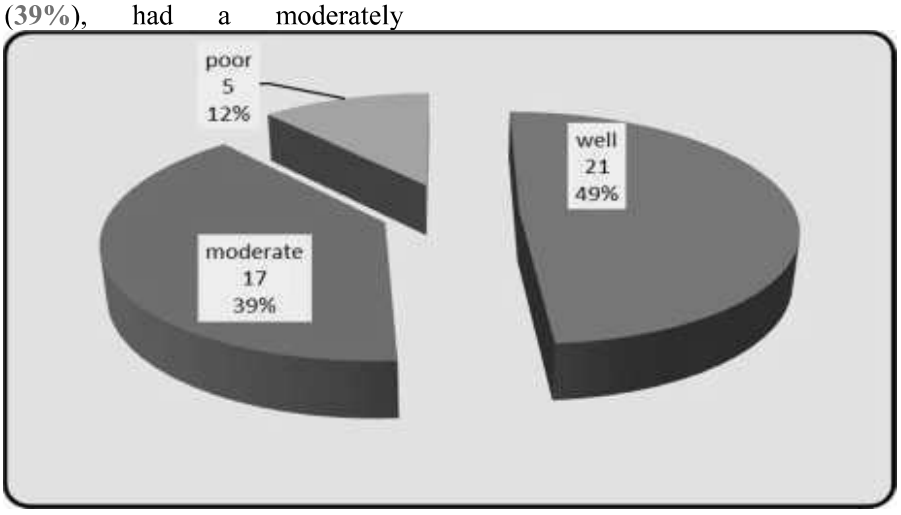
Correlation between pten gene expression and advancing age:

There was a negative correlation between PTEN gene fold change and advancing age. And Despite being positive, this correlation was not significant. The non-parametric Kruskal Wallis test was performed to compare median fold change among different age groups. This revealed a non-significant P-value (0.663). As in Figure below which showed median fold change in various groups.



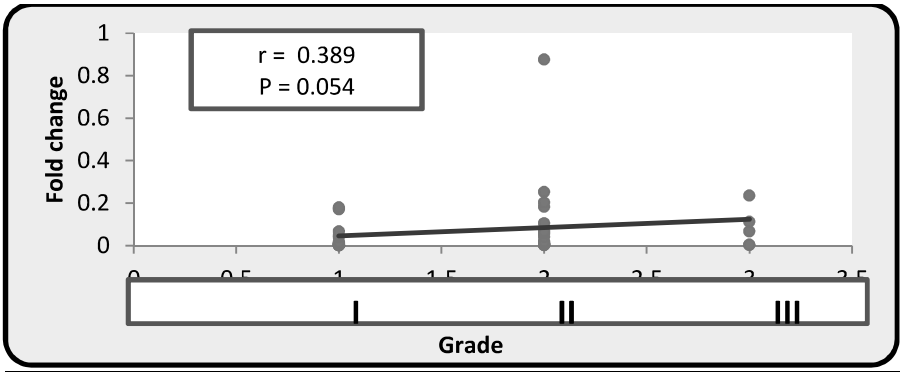
Number of cases classified according to grade

Twenty one cases had a well differentiated endometroid endometrial carcinoma, accounting for (49%). Seventeen cases, (39%), had a moderately differentiated type and. Only (5) cases exhibited a poorly differentiated histologic pattern.

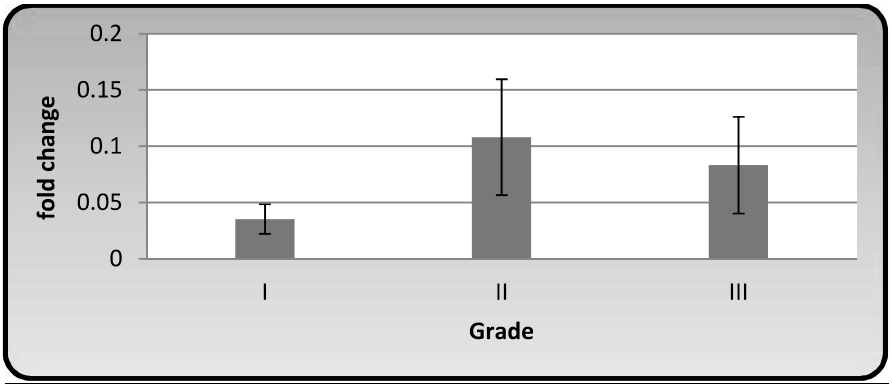


Correlation between PTEN gene fold change and grade of tumor

When fold change was plotted against grade of tumor, the correlation was positive, r = **0.389**. In other words, the higher the grade, the higher PTEN gene fold changes. This correlation carried a borderline significance and P-value was **0.054**.



Mean fold change in different grades was 0.035+0.013, 0.108+ 0.052 and 0.069+0.022 in grade I, II and III respectively. These finding showed in figure below



Discussion

Correlation between pten gene mutation and advancing age:

Our study reveals a negative correlation between pten gene expression and advance age in endometrioid endometrial carcinoma with no significant value Salvesen H. B [9] .stated that PTEN gene mutation correlated with young age group. But Rodrigues M. A [10] Stated that PTEN gene expression was down regulated with advancing age in normal neural tissue of rats. Rocha B. R [11]. Stated that there no significant association between advancing age and PTEN expression in normal uterus of rats. Results of the present study showed a more down regulation of PTEN expression with advancing age, but this was statistically not significant. Even though, patients younger than 45 years of age had the least level of PTEN gene expression. Presence of PTEN gene mutation in young age group patients, as well as old age group patients, suggests that PTEN gene mutation is an early event in the pathogenesis of endometrioid subtype of endometrial carcinoma .

Correlation between Grade of endometrial carcinoma and PTEN gene mutation: PTEN gene mutation were detected in all grade of endometrial carcinoma whether in low or high grade and this is similar to that was observed Anna s. *et al* [12] in their study, this denote to the importance of PTEN gene mutated in initiation of endometrioid carcinogenesis and possibly as an early event in this disease and support this is the study performed by Mutter GL *et al* [13] who found that PTEN gene mutation observed in non-neoplastic endometrium premenopausal women. The our present study we reported borderline negative significance correlation between pten expression and grade, this our result supported by Salvesen *et al* [9] stated that mutation of PTEN gene correlated with low grade endometrial cancer, The same result was reported by Abd EL-MAQSOUD *et al* [14] whose showing a significant negative correlation between PTEN expression and grade was identified particularly between grade 1 and grade 3.the Kimura, *et al* [15] also reported that the PTEN expression higher in grade 3 tumors than in grade 1 and 2 tumors .

Conclusion

PTEN gene mutation was not correlated with advancing age indicating that PTEN gene mutation is an early event in endometroid endometrial carcinoma and an important initiating factor. Other role detected by this study that PTEN gene mutation is a good prognostic factor in endometroid endometrial carcinoma. When PTEN gene mutation was well correlated with grad of tumor, the higher mutation rate associated with lower grad indicating. PTEN gene mutation was not correlated with depth of myometrial invasion, involvement of cervix and different histological stages of tumor indicate that present this mutation an early event in endometroid endometrial carcinoma and not related with cancer development

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الطفرات الوراثية للجين البتين في سرطان بطانة الرحم وعلاقته بالعمر ودرجة تميز السرطان

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الخلاصة

اجريت هذة الدراسة لتقييم تاثير الطفرات الجينية للجين PTEN على الصفات الهستوباثولوجية السريرية لسرطان بطانة الرحم من خلال تقدير العلاقة بين التعبير الجينى للجينPTEN وبعض الخصائص المرضية السريرية (العمر ودرجة تميز السرطان).جمعت ثلاث واربعون عينة من نسيج سرطان بطانة الرحم وعشرون عينة من نسيج غير سرطانى لبطانة الرحم تم الحصول عليها من محتبرات النسيج المرضى العراقية من بداية 2008الى عام 2014 وتم تقدير قيمة التعبير الجينى للجين PTENعن طريق استخلاص الحامض النووى الرايبي الكامل وتوليف الحامض التكميلي للجينPTENتم تضخيمها بجهاز عكس النسخ لتفاعل البوليميراز المتسلسل الكمى وحسبت النتيجة بطريقة ليفاك النسبية .اضهرت نتائج التحليل الاحصائى عدم وجود ارتباط بين تعبير الجين PTENوالتقدم بالسن مما يدل على ان الطفرات الجينية للجينPTEN فى سرطان بطانة الرحم مو جود فى جميع اعمار النساء المصابة بسرطان بطانة الرحم ولا يرتبط بتقدم السن واضهر التحليل ان الطفرات الجينية للبتين موجودة فى جميع درجات الورم ومع وجود ارتباط بين نسبة التعبير الجينى للبتين ودرجات تميز الورم.

الكلمات المفتاحية : جين , سرطان بطانة الرحم .