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A comparison between LncRNA HOTAIRexpression in breast cancer patients and healthy controls due to tumor grade.

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Abstract— Background: In the context of searching for a new molecular markers that contribute to under standing the pathological mechanisms of breast cancer and this aims to develop targeted and promising treatment lines. Several experiment studies have clarified the role of Long non-coding RNA molecules in the occurrence of breast cancer. HOTAIR molecule as LncRNA molecule has been extensively studied as a tumer promoter by controlling of gene expression number of genes related to cancer at several molecular levels. This is done through experimental and interventional studies on breast cancer cell lines and tissues. In this study, we tried to approach its expression level in patient's plasma and compare it to healthy controls. **Materials And Methods:** Gene expression evaluation was performed for HOTAIR molecule in the plasma of 30 patients and compared it with control and this is done by performing a reverse transcription polymerase chain reaction QPCR and the gene expression was evaluated by using Livak's method $2^{\square\square CT}$ Then performing statistical analysis. **Results:** The results, of the study showed statistical significance with a value of $P < 0.05$ for the distinctions between level of gene expression for cases and controls .the result of the study also showed that there is a direct relation between level of gene expression and histological grade of the tumor with a value of $p<0,05$..

Key words; breast cancer , LncRNA,HOTAIR

1 - Introduction:

Breast cancer is the most common types of cancer in women. It causes approximately 15% of cancer deaths in USA and 38% of cancer deaths in Syria in 2020 . Genetic predisposition contributes to a large extent to breast cancer such as BRAC1, BRAC2, EGFR, HER2, TP53 [2, 3] C. MYC mutations.

Long non - coding RNA: Is a class of non - coding RNA molecules, which is long more than 200 nucleotide and It plays an essential role in the development of tumer. [4]
HOX transcript anti sense RNA "HOTAIR" is considered one of the most extensively studied molecules in breast cancer. It consists of 6 axons and is located on chromosome 12 q 13, 13 between HoX 11 and HOX 12 sites[5].

The HOTAIR molecule doesn't express a specific protein but it affects gene expression

through a numberof mechanisms that affect of regulating gene expression such as remodeling chromosome ,chromatin reaction or by controlling miRNA molecules. It was found that the high level of gene expression of the HOTAIR molecule has an important role in a number of malignancies, the most important of which is breast cancer [6, 7].

2-1 Role of HOTAIR in BC:

The HOTAIR molecule works to cause genetic modification and epigenetic changes through several mechanisms, it plays a constructive role by linking protein complexes, such as PRC2 and LSD1. These complexes perform histone modifications such as histone methylation which leads to the silencing of certain genes for example HOXD gene ,which responsible of preventing metastases [8].

It can also play an internal competitive role with miRNA molecules and leads to a reduction of the expression of these molecules. That affects a number of cellular signaling pathways. Which is mediatedby These molecules such as a P27 PENTpath [9].

It can also affect the level of splicing by controlling splicing factors, and it can interferes with translation through it's association with ribosome [10]. through these multiple mechanisms, HOTAIR is controlledin the expression of many genes including regulatory genes for cellular circuit CKD4, CKD6, CYclinD, E2F, Rb. It leads to overcoming the restriction in G1 phase [11] . It also silences many tumor suppressing genes, such as HOXD gene which is located on chromosome 2, It is one of the most important genes that suppress the occurrence of metastases as well as PENT, P21, P53 genes. Each of them works through different mechanism to suppress tumor development [13]. It can enhance the effect of some oncogenic molecules such as HER2 [14], VEGF, Vimentin [15], B-catening,They are all tumor inducing gene [16]. Of all these influences gene expression for HOTAIR molecule closely related to the progression of tumor grade [17] and tumor recurrence [18] and evation of immune system [19] and the occurrence of tumor angiogenesis [20] and drug resistance [21] and the occurrence of metastases, and the inhibition of apoptosis [22] and the occurrence of epithelial mesenchymal transition [23]. for a decade, there have been experimental studies in laboratories to investigate the effect of gene expression level for HOTAIR molecule on cancer mechanisms. There has been interventional studies investing the effect of reducing the expression level on cellular pathways, It was found that Reducing its expression leads to inhibition number of path ways that stimulate tumor formation and it improve therapeutic response to tumor.

It is worth noting that there are several factors in breast cancer that affect the level of HOTAIR expression, Whereas the catalytic regions present in HOTAIR have binding sites for many factors including hormonal factors such as estradiol or Bisphenol or Diclhylstibestrol [24].There are some hormonal components signaling FOXA1 and FOXM1, It can be used for the purpose of reducing HOTAIR expression directly.]25[.there are also sites where some proteins can bind , such as IRF1]26[and transcription factor C-MYC]27[,indicating the mutual role between C-MYC and HOTAIR]28[there was an urgent need to investigate its expression level in clinical trials for the

patients after provingits role in regulating tumor development, in preparation for targeting it therapeutically to contribute toimproving the response to treatment [29].
A few numbers of experiments have begun monitoring their levels in the tissues of breast cancer patients. In our study, we will try to detect its levels in patient's plasma, and comparing it with controls, in addition we are going to detect the relation between high expression levels with tumor grade.

Materials and Methods:

Sample collection:

In this study, samples were collected from 30 patients newly diagnosed with breast cancer, and before performing any therapeutic intervention on the patient and ten healthy controls were taken (negative mammography) and that between August month 2022 and April month 2023. And sampling included inclusion criteria exclusion criteria so the inclusion criteria:
1- Newly diagnosed patient.2- The age over 19 years.
3- Without any therapeutic interference.As for the exclusion criteria were:
1- Without any previous malignancies story.2- Without any chemotherapy story.
3- without autoimmune disease.4- Under the age of 18.

RNA extraction:

After collecting samples on EDTA tubes, the samples were transferred at a speed of 12000 g at a temperature of 4 for 10 minutes and then we transfer the net to the working tubes. The triazol kit, was used to extract the total RNA. This is according to the instructions included in kit. Then concentration and purity of RNA were confirmed by measuring with NARO Drop device.

3-2 CDNA synthesis:

It was used revert AID first strand cDNA where as it was added RNA from the sample to the kit, which contains a primer "Random hexamer primer mix" that works to completely transform the whole RNA into cDNA.

4-2 Quantitative reverse transcription Polymerase chain reaction QPCR:

The expression level of the HOTAIR molecule was evaluated for the patients and for the controls. This is done by performing a qPCR reaction using a Rotrogene device (Qiagen, Germany).

HOTAIR molecular primers were designed:
For word (GGTAGAAAAAGCAACCACGAAGC)reverse
(ACATAAACCTCTGTGTGTGAGTGCC)

primer for GAPDH reference gene were also designed; For word (GTGAAGGTCGGTGTGTGAACGG) reverse (GATGCAGGGATGATGTTCTG)
The ROX qPCR master mix/maxima SYBR green kit was used according to the information mentioned in the kit where the protocol was as following: 10 minutes setting at 95 C° degree then 40 heat cycles for 15 seconds at 95 C° degrees. Then 1 minute at 60 C°, Then perform a melting curve to ensure the quality of PCR products.

Then the gene expression calculation was performed using livak method 2□□CT.

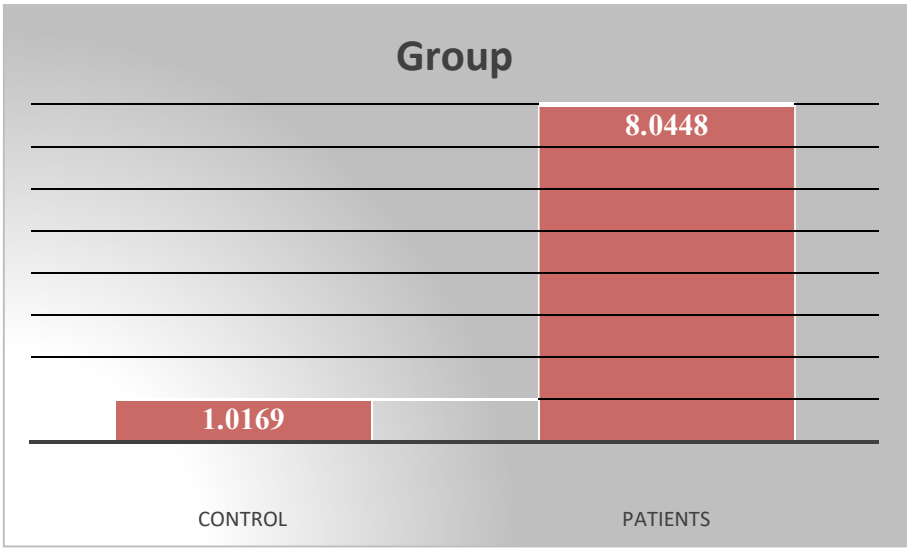
3- Results

The reaction of q PCR was performed to measure level gene expression for 30 patients and 10 healthy controls and the change was calculated.

3-1- The comparison between cases and controls:

	Group Statistics					
Sig. (2-tailed)	Std. Error Mean	Std. Deviation	Mean	N	Group	
.000	1.26098	6.90665	8.0448	30	patients	F-ch
	.05850	.18500	1.0169	10	Control	

The average of change in gene expression (fold change) F-CH for a group of patients reached 8.04 with a standard deviation on 6.90 while the average of controls reached 1.016 with a standard deviation 0.185 and to see if these differences are statistically significant T independent samples test was used where thevalue of statistical significance reached 0.00. it is less than the significance level of 0.05. Therefore, thedifferences are statistically significant.



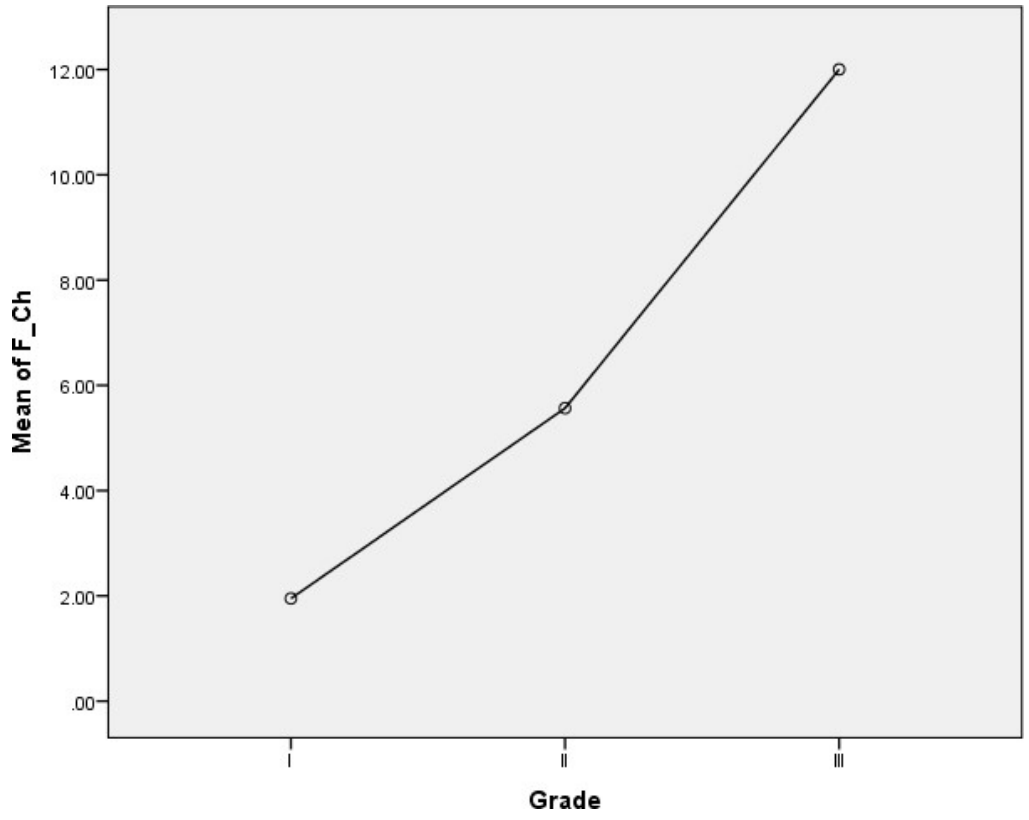
This is consistent with experimental studies conducted on HOTAIR molecule in laboratories on cell linesand cancer tissues. Zhao, W. etal , found that HOTAIR affects apoptosis ,which is considered one of the most preserved processes over the years of development and it is evident in the tissue homeostasis causedby harmony between cell replication and apoptosis. This process is controlled by two types of proteins pro apoptotic like [Bad, Bax] and anti apoptotic like [Bcl-w, Bcl-2] where HOTAIR works to disrupt thisbalance and this is done by

controlling several means where it affects the gene HMGA2 through an axis miR-20-5a, where in the normal state this molecule suppresses gene expression HMGA2 for adults without children and this is because it is one of the main genes contributing to the development and growth of the body, In tumor development , the expression level of HOTAIR increases ,it inhibits the molecule miR-2-5a which leads to increase gene expression of HMGA2 [30]. Ding, W.et al , found HOTAIR also affects the same mechanism on Bel-w by curbing the effect miR-601 and this stimulate the anti-apoptosis gene.[31]

3-2 The comparison at level of gene expression according to the grade:

Sig.	Maximum	Minimum	95% Confidence Interval for Mean		Std. Error	Std. Deviation	Mean	N
			Upper Bound	Lower Bound				
.008	4.50	.90	4.6675	-.7675	.85391	1.70783	1.9500	4
	14.90	.90	7.7996	3.3327	1.02509	3.69603	5.5662	13
	29.00	2.18	16.9978	7.0084	2.29239	8.26534	12.0031	13
	29.00	.90	10.4897	5.2570	1.27924	7.00670	7.8733	30

The average of change at the level of gene expression "F-Ch fold change" for grade group (1) reached 1.9500 with a standard deviation 1.70783 while the average of F-Ch for the grade of group (11) reached 5.5662 with a standard deviation 3.69603 and the average of F-Ch for the grade of group (111) reached 12.0031 with a standard deviation 8.26534 and to determine whether these differences were statistically significant. The ANOVA test was used where the value of statistical significance was reached 0.00 and it is less than the significance level of 0.05, there for the differences are statically significant.



This is consistent with many studies that have demonstrated the role of HOTAIR in preserving cancer cells in the stem state and its role in stimulating epithelial mesenchymal transition EMT. Dittmer, J. et al. found that HOTAIR works to stimulate genes (SNAIL , ZEB1) related to increase incidence of EMT [32]

Deng, J. et al .found that HOTAIR stimulate a group of genes related to cessation of differentiation and maintaining the cells in a stem state like [Sox1, SOX10, OCT4].They become active genes and are repressed by HOTAIR effect across molecules MiR-34a which work to suppress the transcription of these genes These stem cells are identified in breast cancer by the expression [CD 44] on the cell surface and the absence of expression [CD 44+, CD 24-] CD 24 [33].

Lie,K. et al.also mentioned that HOTAIR targets the gene (P53) and affects its connection to stimulate gene transcription P21 which symbolizes for P21 protein which inhibits a number of cycling proteins kinas especially Cd K2 which is responsible for cell transition from phase G1 to phase [34].

Discussion

Through our study, the results were consistent with the role of the HOTAIR molecule as a predictive marker in breast cancer, and the expression level is related to the grade of tumor progression, so the importance of this study comes from of being clinical and on breast

cancer patients it contributes to evaluate its role as a prognostic indicator apart from experimental studies.

Future studies can focus and study the HOTAIR molecule as a therapeutic target via small intervention molecules, which will improve prognosis.

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