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Erythropoietin, FOXA1 And Estrogen Receptors Correlation Is The Key To Understand Pathogenesis Of Breast Cancer

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Abstract

Introduction Erythropoietin is a glycoprotein hormone normally produced by the kidney and fetal liver, acts via erythropoietin receptors to stimulate growth, prevent apoptosis, and induce differentiation of RBC precursors, Expression of EPO and EPOR by tumors of nonhematopoietic tissues may also stimulate cancer. EPOR has no intrinsic kinase activity, it binds and activates intracellular tyrosine kinases to elicit its mitogenic signals. FOXA1 in several studies is cancerogenic. Depletion of FOXA1 protein in MCF-7 breast cancer cells leads to reduced estrogen dependent gene expression and proliferation. **Aim of study.** Study the correlation between Erythropoietin, FOXA1 and estrogen receptors to understand pathogenesis of breast cancer. **material and methods** Ten identical plates cDNA which contain normal and breast cancer with different stages was purchased from OriGene Technologies, Quantitative Real Time Polymerase Chain qRT-PCR was performed with a Rotor-Gene Q PCR (QIAGEN,German), using 2 µL cDNA, 10 µL 2X Sybergreen Master mix (150mM Tris, pH 9.2, 40mM(NH4)2SO4, 5mMMgCl2, 0.02% Tween-20, 0.4mM dNTPs, 1.25 Units Taq Polymerase, 1X Sybergreen) and 0.5 µL of 20µM gene-specific primers (Table 1). **Result** There is a significant difference in Erythropoietin receptor, FOXA1 Estrogen receptors mRNA expression, between normal and patient with breast cancer. Significant positive correction as erythropoietin, FOXA1 and estrogen receptors mRNA expression. discussion they are many of studies in different way confirm the role of Erythropoietin FOXA1 are risk factors for development and progression of Breast cancer and cancer in general. In this study we are going to identify the relation between three component Erythropoietin, FOXA1 and estrogen expression in the same sample to make sure the correlation.

They are strong positive correlation between erythropoietin, and FOXA1 and estrogen mRNA gene expression figure 10 table 13. **Conclusion** Erythropoietin hormone and its receptor is cancerogenic in androgen tissue depending, like prostate gland and breast through activation FOXA1 which in turn increase the activity of number of estrogen receptors expression, erythropoietin and FOXA1 correlation is regard as novel approach therapeutic targeting for breast cancer.

Keywords: Erythropoietin- FOXA1- Estrogen receptors.

الملخص

المقدمة هورمون erythropoietin هو هرمون بروتين سكري يتم انتاجه في الكلية والكبد في فترة حياة الجنين . يحفز النمو ويثبط موت الخلايا ايضا يعمل على تحفيز انتاج خلايا الدم الحمراء يعد انتاج هرمون erythropoietin في الانسجة الغير مصنعة للدم عامل مسرطن. يعد FOXA1 في العدد من الدراسات السابقة ايضا من العوامل المسرطنة. **هدف الدراسة** تهدف الدراسة للبحث العلاقة ما بين ال erythropoietin و FOXA1 في تحفيز انتاج مستقبلات estrogen . **المواد وطرق القياس** 10 من قوالب cDNA لأشخاص طبيعيين و مرضي سرطان الثدي بمختلف اطوار مرض سرطان الثدي جلبت من شركة OriGene بالولايات المتحد الأمريكية بعد ذلك تم تحديد primers المناسب لقياس mRNA لكل من erythropoietin و FOXA1 estrogen receptors عبر Fast Real-Time PCR System بنظام الصبغة الخضراء Sybergreen Master mix حسب البروتكول الموصي به . **النتائج** هنالك زيادة في مستقبلات هرمون ال erythropoietin و FOXA1 و ال estrogen receptors فى مرضي سرطان الثدي مقارن مع الاشخاص الطبيعيين تم بعد ذلك بحث في ما اذا كان هنالك علاقة ارتباط بين كل من erythropoietin و FOXA1 و estrogen receptors وكانت النتائج كما يلى هنالك علاقة إيجابيه خطية قوية بين العناصر الثلاثة erythropoietin , FOXA1 و مستقيلات ال estrogen كما هو موضح في الشكل 100 و جدول 13 **الختام و توصيات البحث** من خلال نتائج البحث يعد ال erythropotein في الانسجة المعتمدة على هرمون الأستروجين عامل مسرطن و يعتمد في عملة على تحفيز ال FOXA1 الذي بدورة يذيد من تكوين مستقبلات الاستروديين.

الكلمات المفتاحية مستقبلات هرمون الارثروبيوتين – فوكس اي 1 – مستقيلات الاستروجين.

Introduction

EPO,4 a glycoprotein hormone normally produced by the kidney and fetal liver, acts via EPORs to stimulate growth, prevent apoptosis, and induce differentiation of RBC precursors (Miura, D'Andrea, Kabat, & Ihle, 1991). Expression of EPO and EPOR has recently been demonstrated in several nonhematopoietic tissues (Acs et al., 2001), which suggests broader roles for EPO signaling in regulating cell growth, cell survival, and angiogenesis (Acs et al., 2001; Yasuda et al., 2001). Expression of EPO and EPOR by tumors of nonhematopoietic tissues may also stimulate cancer. EPOR has no intrinsic kinase activity, it binds and activates intracellular tyrosine kinases to elicit its mitogenic signals (Miura et al., 1991; Robinson et al., 2006) Autocrine/paracrine erythropoietin (EPO) action, promoting cell survival and mediated by its receptor (EPOR) in various solid tumors, including breast carcinoma **(**Pelekanou et al., 2007)Estrogen plays an important role in the growth, proliferation, and differentiation of mammary epithelium. ERa and Erh mediate the biological action of estrogen by functioning as estrogen-activated transcription factors (Ali & Coombes, 2002; Deroo & Korach, 2006). ERa is expressed in 10% to 15% of luminal epithelial cells of normal breast and these cells are generally considered slowly proliferating and well-differentiated cell types **(11)**. However, >50% of breast cancers express ERa at the time of initial diagnosis(Ali & Coombes, 2002) · The expression of EPO/EPOR is steroid dependent in some tissues; however, a clear relationship of EPO/EPOR and steroid receptors in breast cancer **(**Pelekanou et al., 2007).In female reproductive organs, EPO/EPOR expression are regulated by estrogen and/or progesterone (Fairchild Benyo & Conrad, 1999; Juul, Yachnis, & Christensen, 1998). EPOR knockdown decreased ERα activity further supports a mechanism by which EPOR affects proliferation via ERαmediated mechanisms.(Reinbothe et al., 2014) FOXA1(forkhead box transcription factor) is also consistently expressed in luminal breast cancer cell(Bernardo et al., 2013) FOXA1 is a ‘‘winged helix’’ transcription factor, which has recently been dubbed as a ‘‘pioneer factor’’ responsible for the recruitment of ERa to the genome(Carroll & Brown, 2006).

Depletion of FOXA1 protein in MCF-7 breast cancer cells leads to reduced estrogen dependent gene expression and proliferation, which is consistent with its role in mediating the effects of estrogen (Carroll et al., 2005; Laganière et al., 2005). The COOH-terminal region of FOXA1 interacts with histones H3 and H4 and this interaction is responsible for opening compacted chromatin. By opening chromatin, FOXA1 may permit efficient interaction of ERa with its response elements and subsequent interaction of ERa associated histone modifying enzymes with histones. Consistent with this possibility, about half of estrogen-regulated genes contain binding sites for FOXA1 (Carroll et al., 2005)· Optimum expression of these estrogen regulated genes may occur only in cells that co express Era and FOXA1 and only these cells may be addicted to estrogen dependent survival and proliferation signaling pathways. Thus, mammalian forkhead transcription factors are involved in EPO signaling in primary erythroid progenitors and may play a role in the induction of apoptotic and mitogenic signals.(Mahmud et al., 2002)

literature review

Erythropoietin drives breast cancer progression by activation of activation of the PI3K/AKT and MAPK pathways

Breast cancer is the number one killer of cancer. Anemia is frequent in breast cancer patients and can be treated by blood transfusions or intravenously erythropoietin (EPO) to encourage the production of red blood cells. Clinical studies have shown declines in survival in some groups of cancer patients being treated with EPO. Many tumor cells express the EPO receptor (EPOR), posing a risk that EPO treatment would increase the tumor's growth, but the mechanisms involved in the progression of the mammary tumor are misunderstood. EPO triggered the activation of the PI3K/AKT and MAPK pathways in human breast cancer cell lines. EPOR Down Regulation inhibits excessive growth of human tumor cells. induced apoptosis by Bim, decreased invasiveness, and caused degradation of MYC expression.

The EPO-induced expression MYC is transmitted by the PI3K/AKT and MAPK routes, and overexpression of MYC is partially saved loss of cell proliferation caused by EPOR downregulation. In a xenotransplantation model, designed to simulate recombinant EPO treatment in breast cancer patients, reversal of EPOR significantly reduced tumor growth.(Chan et al., 2017)

Serum erythropoietin levels, breast cancer and breast cancer-initiating cells

Cancer is often associated with tumor-related anemia, and many chemotherapy agents interfere with hematopoiesis, which affects quality of life for affected patients. Cancer is frequently associated with tumor-related anemia, and many chemotherapeutic agents impair hematopoiesis, leading to impaired quality of life for affected patients. The use of erythropoiesis-stimulating agents was investigated after prospective clinical trials using recombinant erythropoietin to correct anemia reported increased incidence of thromboembolic events and deaths due to cancer.(Bhat et al., 2019)

The Forkhead box A1 protein (FOXA1) is a pioneering factor in the binding and α (RE) function of the oestrogen receptor However, the role of FOXA1 in breast cancer and the underlying molecular mechanisms remain unclear.(Jing, Liang, Hao, Hongxia, & Cui, 2019)

The role of forkhead-box A1 (FOXA1) and Androgen receptor (AR) in breast cancer (BC) has been extensively studied. However, the prognostic role of their co-expression in Estrogen receptor positive (ER+) BC has not been investigated so far.(Rangel et al., 2018)

FOXA1 augmentation, including by genetic aberrations, drives aggressive phenotypes of estrogen receptor-positive (ER+) breast cancer (BC). Here, we show that FOXA1 upregulation induces genome-wide enhancer reprogramming and adopts a superenhancer mechanism to activate the master transcription factor HIF-2α and a prometastatic transcriptional program.(Fu et al., 2019)

Down regulation FOXA1 in luminal MCF-7 and T47D cells, we found an enhance in doxorubicin and paclitaxel sensitivity as well as a decrease in anchorage independence. And FOXA1 up-regulation of in basal-like MDA-MB-231 cells led to an increase in drug resistance and anchorage independence.(Kumar, Ardasheva, Mahmud, Coombes, & Yagüe, 2021).

In this study according to literature review, we are going to identify the relation between the erythropoietin receptor mRNA expression FOXA1 and estrogen receptors mRNA expression **(**study question or hypothesis's,**)** ***Erythropoietin, FOXA1 and estrogen receptors Correlation is the Key to understand pathogenesis of breast cancer.***

Justification

breast cancer is the most diagnosed cancer among women in the U.S. In 2021, there will be an estimated 281,550* new cases of invasive breast cancer diagnosed in women; 2,650* cases diagnosed in men and an additional 49,290 cases of ductal carcinoma in situ (DCIS)** diagnosis in women. (ACS, 2021)

Breast cancer is the number one killer of cancer. Anemia is frequent in breast cancer patients and can be treated by blood transfusions or intravenously erythropoietin (EPO) to encourage the production of red blood cells. Clinical studies have shown declines in survival in some groups of cancer patients being treated with EPO. Many tumor cells express the EPO receptor (EPOR), posing a risk that EPO treatment would increase the tumor's growth.

Objectives

A. Primary objectives

Study the correlation between Erythropoietin, FOXA1 and estrogen receptors to understand pathogenesis of breast cancer.

B. Secondary objective

1. Study erythropoietin receptors mRNA gene expression in normal subject and patent with breast cancer in different stages .
2. Study FOXA1 mRNA gene expression in normal subject and patent with breast cancer in different stages.
3. Study estrogen receptors mRNA gene expression in normal subject and patent with breast cancer in different stages.

Research questions

How the Erythropoietin is carcinogenic is it any correlation between Erythropoietin and FOXA1 in estrogen receptor expression regulation

Materials and methods

Samples

Ten identical plates cDNA which contain normal and breast cancer with different stages was purchased from OriGene Technologies, Inc. 9620 Medical Center Drive

Suite 200 Rockville, MD 20850 USA.

Methods for Quantitative Real Time Polymerase Chain

qRT-PCR was performed with a Rotor-Gene Q PCR (QIAGEN,German), using 2 µL cDNA, 10 µL 2X Sybergreen Master mix (150mM Tris, pH 9.2, 40mM(NH4)2SO4, 5mMMgCl2, 0.02% Tween-20, 0.4mM dNTPs, 1.25 Units Taq Polymerase, 1X Sybergreen) and 0.5 µL of 20µM gene-specific primers (Table 1). Primers were designed based on theoretical optimal conditions, which included primer melting temperature, primer annealing temperature, GC content, cross homology and primer secondary structures. All primers were purchased from Bio-Basic Canada Inc. (Ontario, Canada). The specificity and size of the PCR products were tested by adding a melt curve at the end of the amplifications, analysis on a 2% agarose gel of the bands. Amplicon Bands were isolated and sequenced. The reaction protocol consisted of one activation cycle of 50◦C for 2min followed by 95◦C for 15 s. Thereafter, 40 cycles of denaturation at 95◦C for 15 s, and at 60◦C annealing/extension for 2min were performed. Although normalization to RPL13 and Ubiquitin C showed similar trends, all values were normalized to Ubiquitin C. The $2^{-\Delta\Delta CT}$ method was used for relative quantification for qRT-PCR experiments.

Table 1 erythropoietin, FOXA1 and estrogen primers

Primers	Forward	Reverse	Accession number
EPOR	TGGAGGACTTGGTGTGTTTCT	GCAACTCTAGGGGCACGAA	NM_000121
FOXA1	GCAATACTCGCCTTACGGCT	TACACACCTTGGTAGTACGCC	NM_004496
Estrogen	GCCGGAATGCAAAGGATGTG	AGGAACCATAAGGAACCTGTC	NM_005420

- **Type of study**

Cross sectional study

- **Statistical Analysis**

Statistical analysis was carried out using Spss software ver.20. Fold change in mRNA expression was calculated for qRT-PCR results and analysis was carried out using One Way ANOVA followed by (t test) for pairwise comparisons and comparisons against the Normal group.

- **Ethical consideration**

All samples labeled with letters and number then distributed randomly in Rt PCR plate to avoid and prevent any subject to use the experimental data.

- **Ethical approval**

Study is part of PhD was approved postgraduate study (University of Bakhtalruda)

college of medicine department of physiology 27/9/2018.

- **Contributions**

Mohand Hassan Moalla Khder designed the study. Experiments, data collection and analysis were performed by, prof. *Amal Seed, Mohand Hassan Moalla Khder*. The first draft of the manuscript was written by *Mohanad Moalla Mowafag Khedir* and, all authors read and reviewed and approved the final manuscript.

- **Conflicts of interest**

The authors declare no conflict of interest.

- **Results**

The main objective is Study the correlation between Erythropoietin, FOXA1 and estrogen receptors to understand pathogenesis of breast cancer.

To achieve this gool we have experimental steps.

Step one study FOXA1, Erythropoietin receptors and estrogen mRNA gene expression in normal subject and patient with breast cancer in different stages in the same sample to avoid any effect of physiological factors like age and Body mass index. Through one way ANOVA table and regression .

Step two study the correlation between Erythropoietin receptors expression, FOXA1 mRNA expression and estrogen receptors expression through bivariate correction .

Erythropoietin receptor mRNA gene expression in normal subject compared to patient with breast cancer in different stages.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
Normal		5	-17.2100	15.83686	7.08246	-36.8741	2.4541	-43.92	-2.75	
Stage I		11	.3527	3.72168	1.12213	-2.1475	2.8530	-2.80	10.69	
Stage IIA		14	2.6007	4.20792	1.12461	.1711	5.0303	-2.54	14.53	
Stage IIIA		14	4.6521	12.37253	3.30670	-2.4915	11.7958	-2.32	43.48	
Stage IV		4	43.3200	96.03030	48.01515	-109.4856	196.1256	-11.39	187.17	
Total		48	4.0135	29.04167	4.19180	-4.4193	12.4464	-43.92	187.17	
Model	**Fixed Effects**			26.86201	3.87720	-3.8056	11.8327			
	Random Effects				7.26660	-16.1618	24.1889			157.08303

Table 2:- Descriptive analysis Erythropoietin mRNA expression

Table 3:- ANOVA Erythropoietin mRNA expression

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8613.256	4	2153.314	2.984	.029
Within Groups	31027.409	43	721.568		
Total	39640.665	47			

Overall, there is significant difference between normal subject and patient with breast cancer. There is a significant difference in Erythropoietin receptor mRNA expression, between normal and patient with breast cancer at the p<.05 level for the three conditions [F (4) = 2.984, *p =* 029*].*

Table 4:- LSD Multiple Comparisons Erythropoietin mRNA expression

(I) Breast cancer Stages	(J) Breast cancer Stages	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Normal	Stage I	-17.56273	14.48829	.232	-46.7812	11.6557
	Stage IIA	-19.81071	13.99479	.164	-48.0339	8.4125
	Stage IIIA	-21.86214	13.99479	.126	-50.0853	6.3610
	Stage IV	-60.53000*	18.01959	.002	-96.8700	-24.1900
Stage I	Normal	17.56273	14.48829	.232	-11.6557	46.7812
	Stage IIA	-2.24799	10.82301	.836	-24.0747	19.5787
	Stage IIIA	-4.29942	10.82301	.693	-26.1261	17.5273
	Stage IV	-42.96727*	15.68404	.009	-74.5971	-11.3374
Stage IIA	Normal	19.81071	13.99479	.164	-8.4125	48.0339
	Stage I	2.24799	10.82301	.836	-19.5787	24.0747
	Stage IIIA	-2.05143	10.15289	.841	-22.5267	18.4238
	Stage IV	-40.71929*	15.22933	.011	-71.4322	-10.0064
Stage IIIA	Normal	21.86214	13.99479	.126	-6.3610	50.0853
	Stage I	4.29942	10.82301	.693	-17.5273	26.1261
	Stage IIA	2.05143	10.15289	.841	-18.4238	22.5267
	Stage IV	-38.66786*	15.22933	.015	-69.3807	-7.9550
Stage IV	Normal	60.53000*	18.01959	.002	24.1900	96.8700
	Stage I	42.96727*	15.68404	.009	11.3374	74.5971
	Stage IIA	40.71929*	15.22933	.011	10.0064	71.4322
	Stage IIIA	38.66786*	15.22933	.015	7.9550	69.3807

*. The mean difference is significant at the 0.05 level.

There is significant difference between Normal and breast cancer stage IV (high erythropoietin mRNA expression in patients with breast cancer compared to the normal)

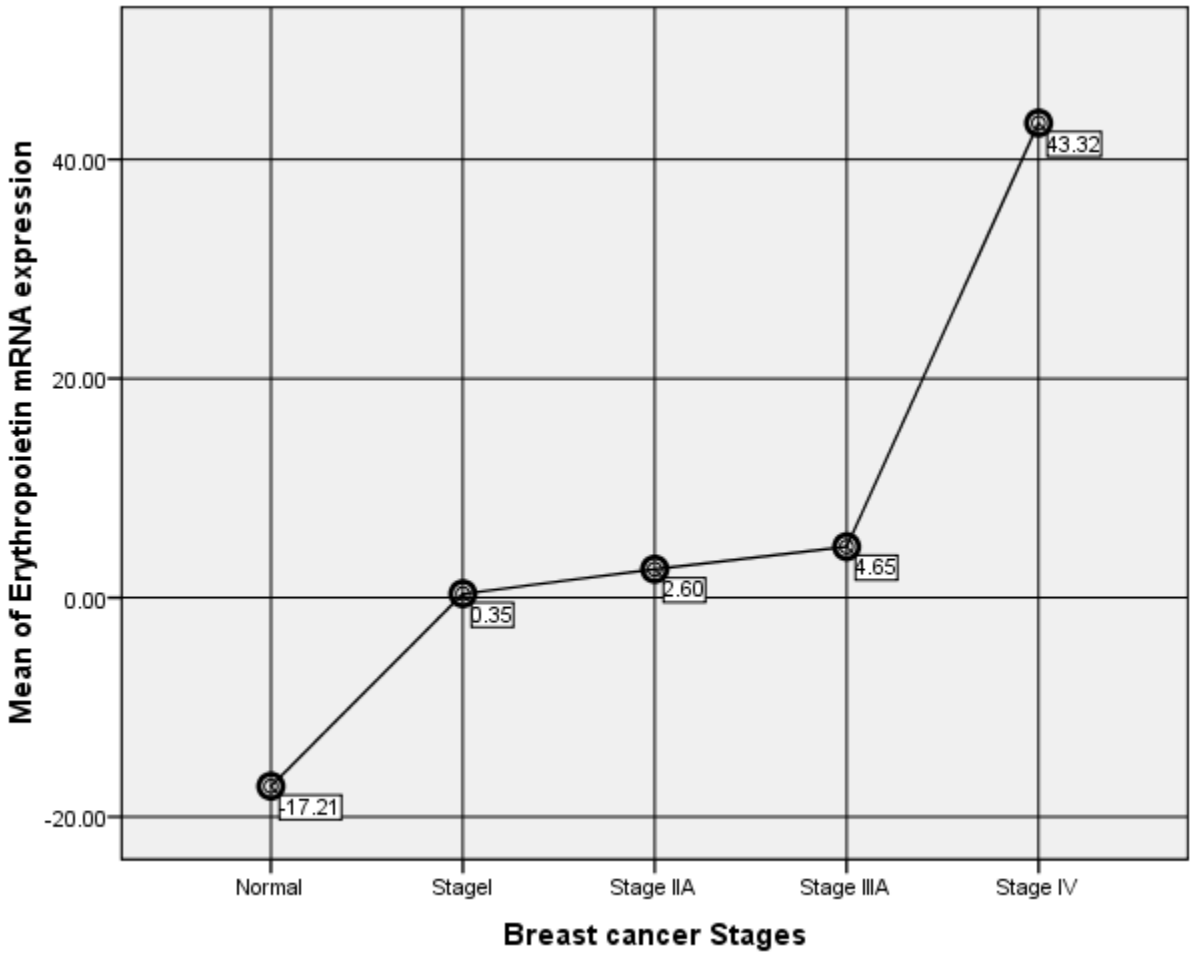

Figure 1:- erythropoietin mRNA expression in patients with breast cancer compared to the normal

FOXA1 gene expression in normal subject compared to patient with breast cancer in different stages.

Table 5:- Descriptive analysis FOXA1 mRNA gene expression

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
Normal		5	-36.0360	20.18136	9.02538	-61.0945	-10.9775	-61.31	-10.15	
Stage I		11	-1.1100	8.21416	2.47666	-6.6283	4.4083	-21.56	10.83	
Stage IIA		14	-1.8707	23.50007	6.28066	-15.4393	11.6978	-78.64	20.11	
Stage IIIA		14	.9721	7.44005	1.98844	-3.3236	5.2679	-20.48	10.11	
Stage IV		4	9.2050	6.87847	3.43924	-1.7402	20.1502	.66	17.26	
Total		48	-3.5031	18.82921	2.71776	-8.9706	1.9643	-78.64	20.11	
Model	**Fixed Effects**			15.51047	2.23874	-8.0180	1.0117			
	Random Effects				6.35124	-21.1370	14.1307			1.46916E2

Table 6:- ANOVA table FOXA1 mRNA gene expression

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6318.622	4	1579.656	6.566	.000
Within Groups	10344.715	43	240.575		
Total	16663.337	47			

There is a significant difference in FOXA1 expression, between normal and patient with breast cancer at the p<.05 level for the three conditions [F (4) = 6.566*, p = 0.000].*

Table 7:- LSD Multiple Comparisons FOXA1 mRNA expression

(I) Breast cancer Stages	(J) Breast cancer Stages	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Normal	Stage I	-34.92600*	8.36573	.000	-51.7971	-18.0549
	Stage IIA	-34.16529*	8.08077	.000	-50.4617	-17.8689
	Stage IIIA	-37.00814*	8.08077	.000	-53.3046	-20.7117
	Stage IV	-45.24100*	10.40474	.000	-66.2242	-24.2578
Stage I	Normal	34.92600*	8.36573	.000	18.0549	51.7971
	Stage IIA	.76071	6.24935	.904	-11.8423	13.3637
	Stage IIIA	-2.08214	6.24935	.741	-14.6852	10.5209
	Stage IV	-10.31500	9.05616	.261	-28.5785	7.9485
Stage IIA	Normal	34.16529*	8.08077	.000	17.8689	50.4617
	Stage I	-.76071	6.24935	.904	-13.3637	11.8423
	Stage IIIA	-2.84286	5.86241	.630	-14.6655	8.9798
	Stage IV	-11.07571	8.79361	.215	-28.8097	6.6583
Stage IIIA	Normal	37.00814*	8.08077	.000	20.7117	53.3046
	Stage I	2.08214	6.24935	.741	-10.5209	14.6852
	Stage IIA	2.84286	5.86241	.630	-8.9798	14.6655
	Stage IV	-8.23286	8.79361	.354	-25.9669	9.5012
Stage IV	Normal	45.24100*	10.40474	.000	24.2578	66.2242
	Stage I	10.31500	9.05616	.261	-7.9485	28.5785
	Stage IIA	11.07571	8.79361	.215	-6.6583	28.8097
	Stage IIIA	8.23286	8.79361	.354	-9.5012	25.9669

*. The mean difference is significant at the 0.05 level.

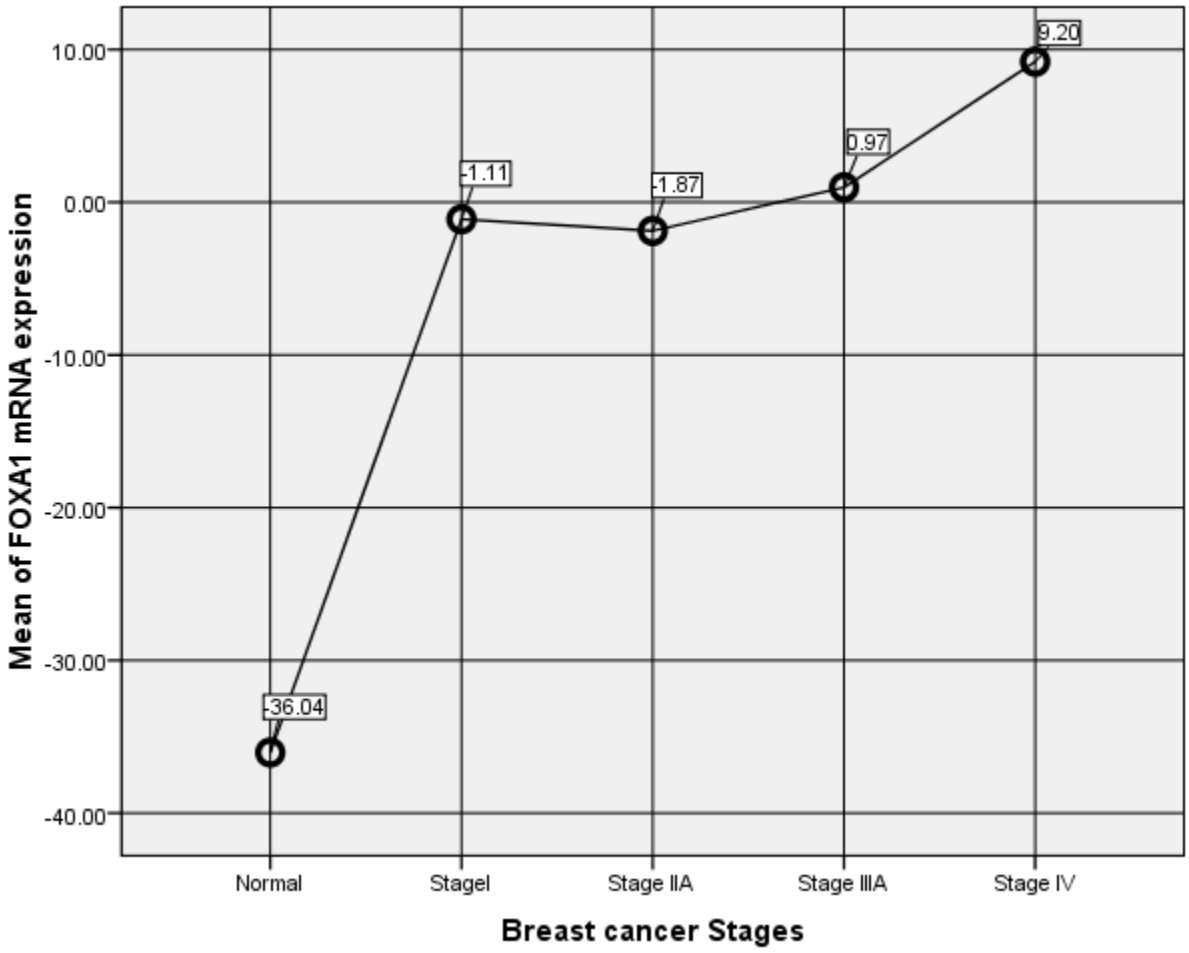

Figure 2:- FOXA1 mRNA gene expression normal subject compared to patient with breast cancer in different stages.

Estrogen receptors m RNA gene expression normal subject compared to patient with breast cancer in different stages.

Table 8:- Descriptive analysis estrogen receptor mRNA gene expression

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
Normal		5	-3.3422E1	3.56215E1	1.59304E1	-7.7652E1	1.0808E1	-92.94	-8.62	
Stage I		11	-.5573	1.87057E0	.56400	-1.8139E0	.6994	-4.91	1.19	
Stage IIA		14	2.2757	7.06740E0	1.88884E0	-1.8049E0	6.3563	-7.79	24.12	
Stage IIIA		14	.5657	1.29421E0	.34589	-.1815	1.3130	-1.60	2.42	
Stage IV		4	6.6728E1	5.71855E1	2.85927E1	-2.4267E1	1.5772E2	17.66	126.96	
Total		48	2.7802	2.86939E1	4.14161E0	-5.5516E0	1.1112E1	-92.94	126.96	
Model	**Fixed Effects**			1.90423E1	2.74852E0	-2.7627E0	8.3231			
	Random Effects				1.22625E1	-3.1266E1	3.6826E1			5.93947E2

Table 9**:-** ANOVA Estrogen receptors m RNA gene expression normal subject compared to patient with breast cancer in different stages.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23104.769	4	5776.192	15.930	.000
Within Groups	15592.194	43	362.609		
Total	38696.963	47			

There is a significant difference in estrogen receptor mRNA expression, between normal and patient with breast cancer at the p<.05 level for the three conditions [F (4) = 2.984, p = 029].

Table 10:- LSD Multiple Comparisons Estrogen receptors mRNA gene expression normal subject compared to patient with breast cancer in different stages.

(I) Breast cancer Stages	(J) Breast cancer Stages	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Normal	Stage I	-32.86473*	10.27065	.003	-53.5775	-12.1520
	Stage IIA	-35.69771*	9.92081	.001	-55.7049	-15.6905
	Stage IIIA	-33.98771*	9.92081	.001	-53.9949	-13.9805
	Stage IV	-100.14950*	12.77396	.000	-125.9107	-74.3883
Stage I	Normal	32.86473*	10.27065	.003	12.1520	53.5775
	Stage IIA	-2.83299	7.67236	.714	-18.3058	12.6398
	Stage IIIA	-1.12299	7.67236	.884	-16.5958	14.3498
	Stage IV	-67.28477*	11.11831	.000	-89.7070	-44.8626

Stage IIA	Normal	35.69771*	9.92081	.001	15.6905	55.7049
	Stage I	2.83299	7.67236	.714	-12.6398	18.3058
	Stage IIIA	1.71000	7.19731	.813	-12.8048	16.2248
	Stage IV	-64.45179*	10.79597	.000	-86.2239	-42.6796
Stage IIIA	Normal	33.98771*	9.92081	.001	13.9805	53.9949
	Stage I	1.12299	7.67236	.884	-14.3498	16.5958
	Stage IIA	-1.71000	7.19731	.813	-16.2248	12.8048
	Stage IV	-66.16179*	10.79597	.000	-87.9339	-44.3896
Stage IV	Normal	100.14950*	12.77396	.000	74.3883	125.9107
	Stage I	67.28477*	11.11831	.000	44.8626	89.7070
	Stage IIA	64.45179*	10.79597	.000	42.6796	86.2239
	Stage IIIA	66.16179*	10.79597	.000	44.3896	87.9339

*. The mean difference is significant at the 0.05 level.

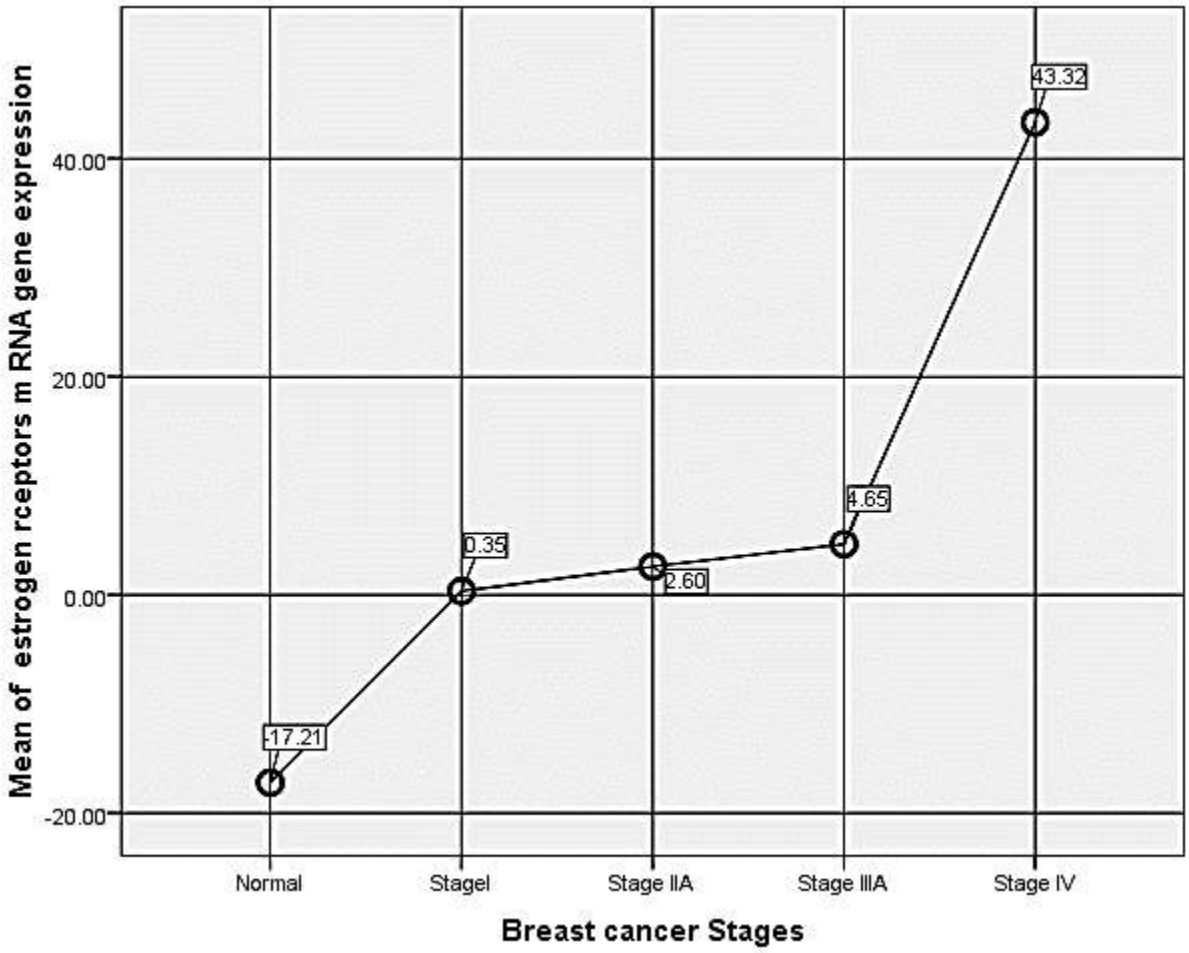

Figure 3:- Estrogen receptors mRNA gene expression normal subject compared to patient with breast cancer in different stages.

Study the correlation between erythropoietin and FOXA1

Table 11 :- Correlations between erythropoietin and FOXA1

		Erythropoietin mRNA expression	FOXA1 mRNA expression
Erythropoietin mRNA expression	Pearson Correlation	1	.248*
	Sig. (1-tailed)		.044
	N	48	48
FOXA1 mRNA expression	Pearson Correlation	.248*	1
	Sig. (1-tailed)	.044	
	N	48	48

**. Correlation is significant at the 0.05 level (1-tailed).*

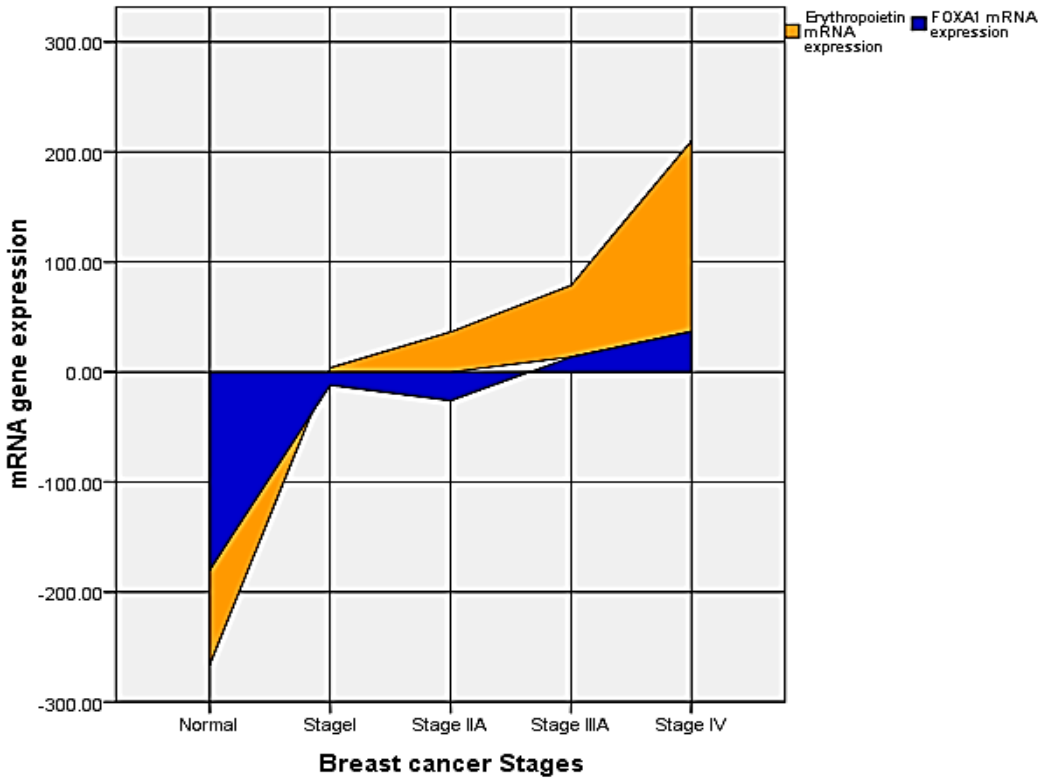

Figure 4:- correlation between erythropoietin and FOXA1

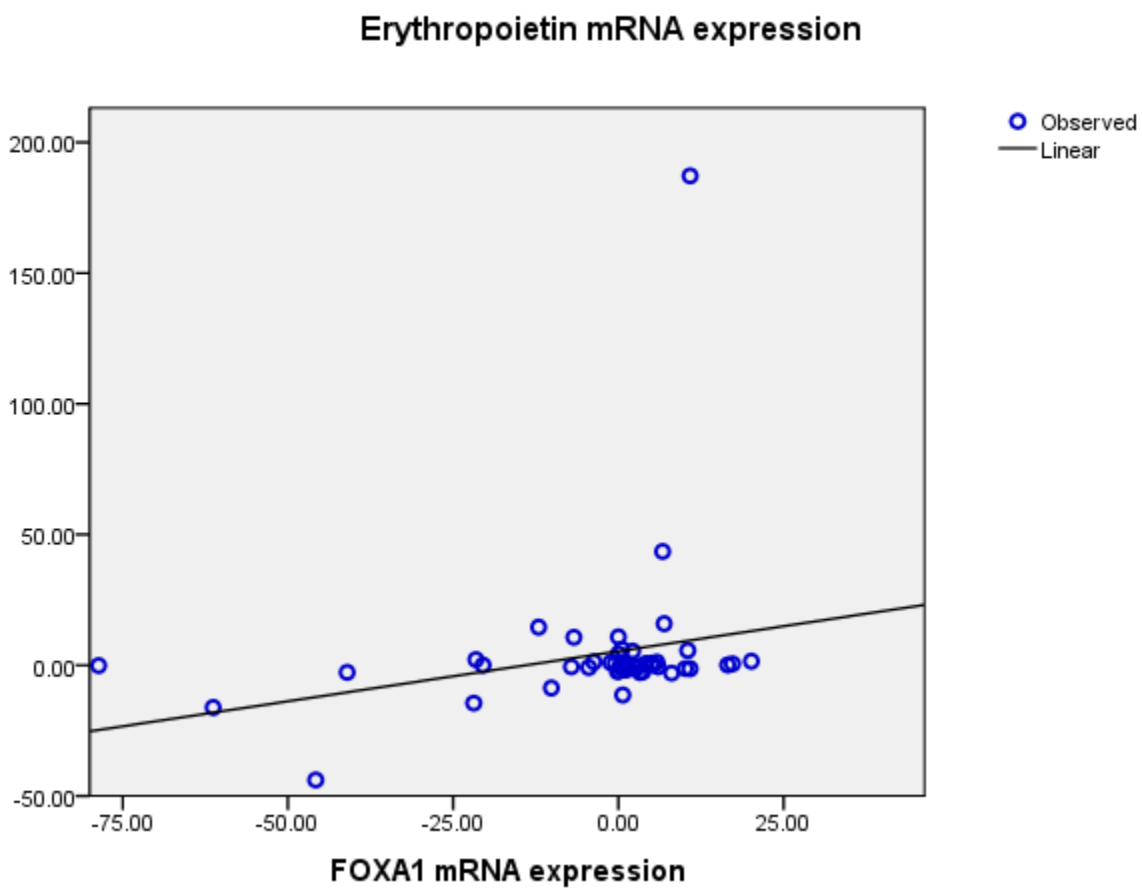

Figure 5: - significant positive correction as erythropoietin mRNA expression increase increases the FOXA1 mRNA expression increase.

Study the correlation between erythropoietin and Estrogen.

Table 12:- Correlations between erythropoietin and Estrogen.

		Erythropoietin mRNA expression	Estrogen receptor mRNA expression
Erythropoietin mRNA expression	Pearson Correlation	1	1.000**
	Sig. (2-tailed)		.000
	N	48	48
Estrogen receptor mRNA expression	Pearson Correlation	1.000**	1
	Sig. (2-tailed)	.000	
	N	48	48

***. Correlation is significant at the 0.01 level (2-tailed).*

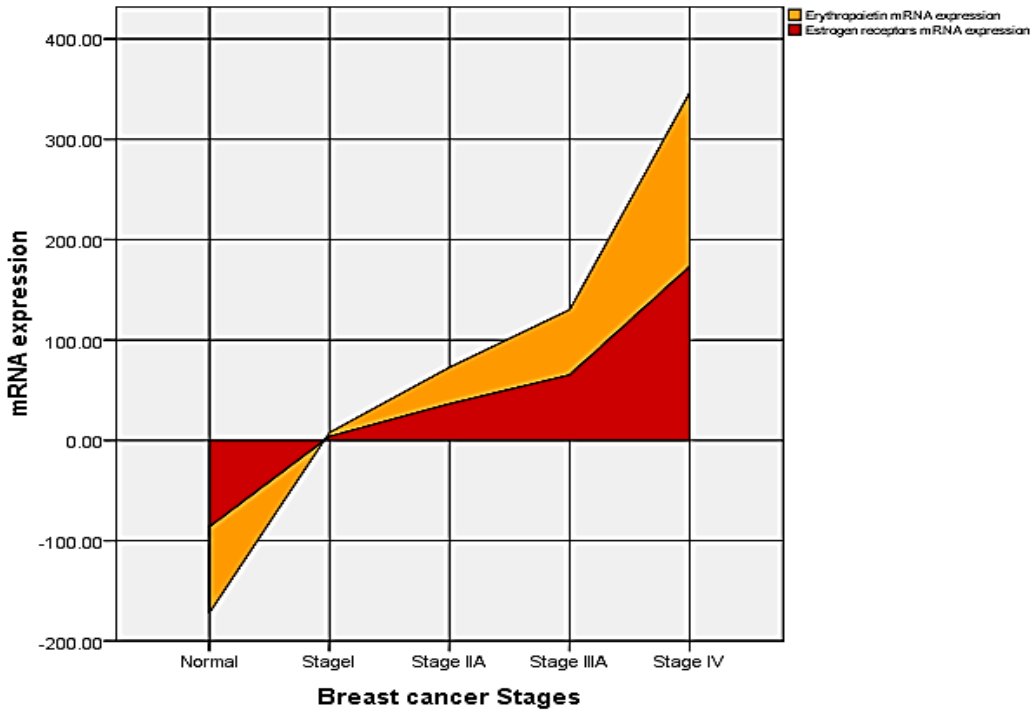

Figure 5 correlation between erythropoietin and Estrogen.

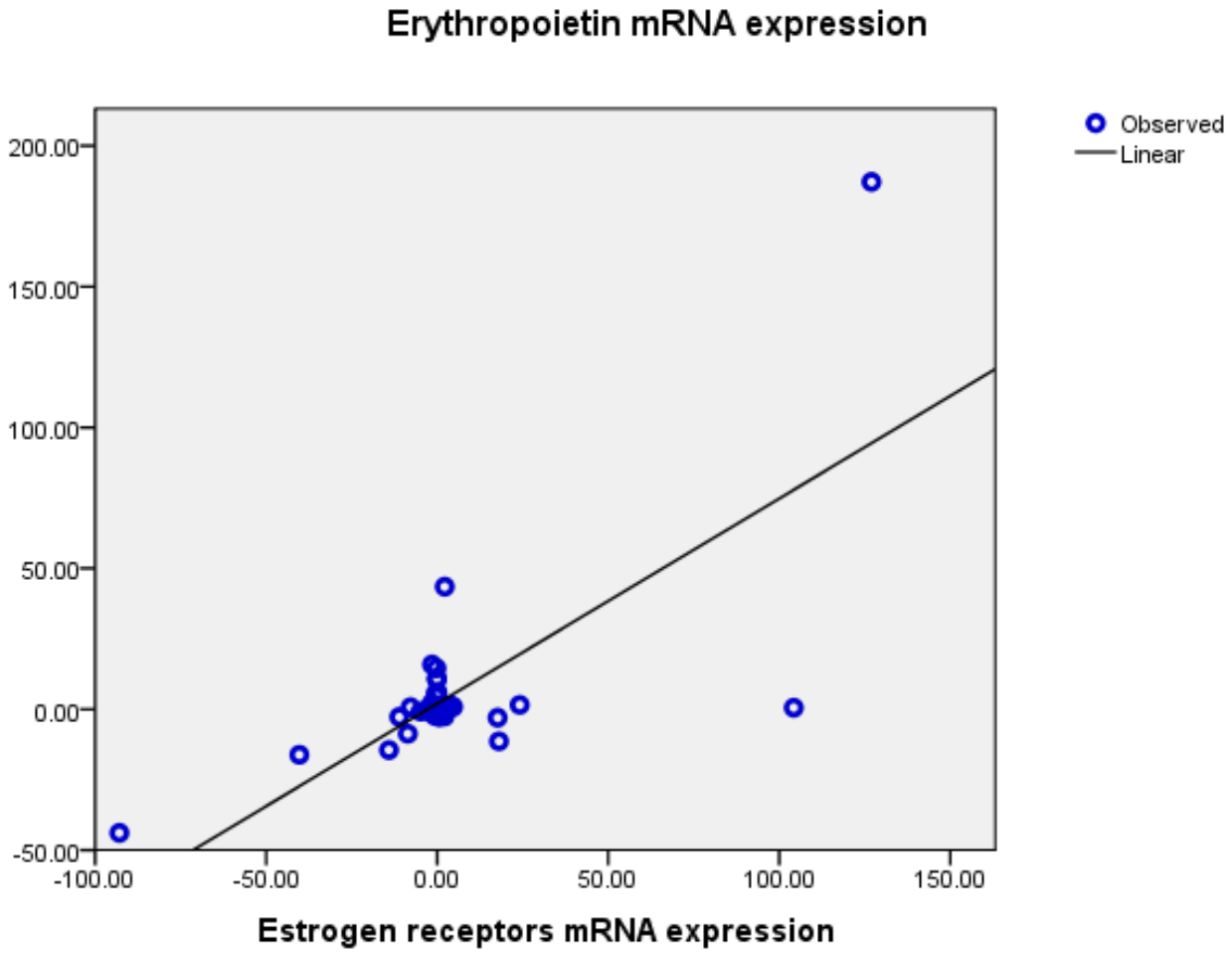

Figure 6 significant positive correction as erythropoietin mRNA expression increase increases the estrogen mRNA expression increase.

Study the correlation between FOXA1 and Estrogen.

Table 13 Correlation between FOXA1 and Estrogen**.**

		FOXA1 mRNA expression	**Estrogen receptors mRNA expression**
FOXA1 mRNA expression	Pearson Correlation	1	.483**
	Sig. (2-tailed)		.000
	N	48	48
Estrogen receptors mRNA expression	Pearson Correlation	.483**	1
	Sig. (2-tailed)	.000	
	N	48	48

***. Correlation is significant at the 0.01 level (2-tailed).*

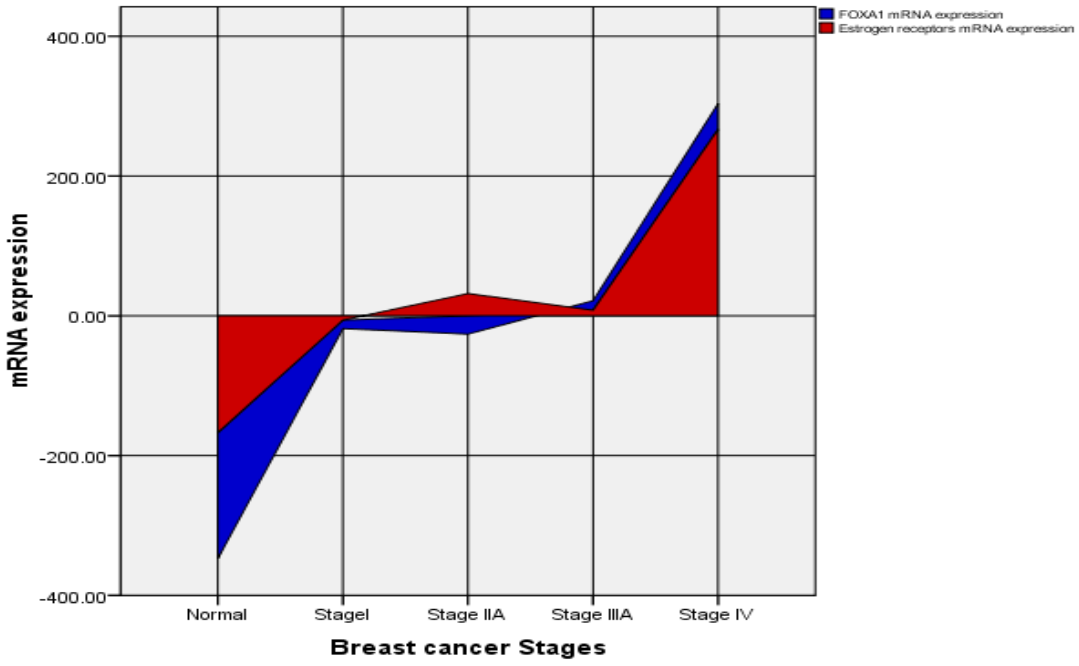

Figure 7 correlation between FOXA1 and estrogen receptors expression

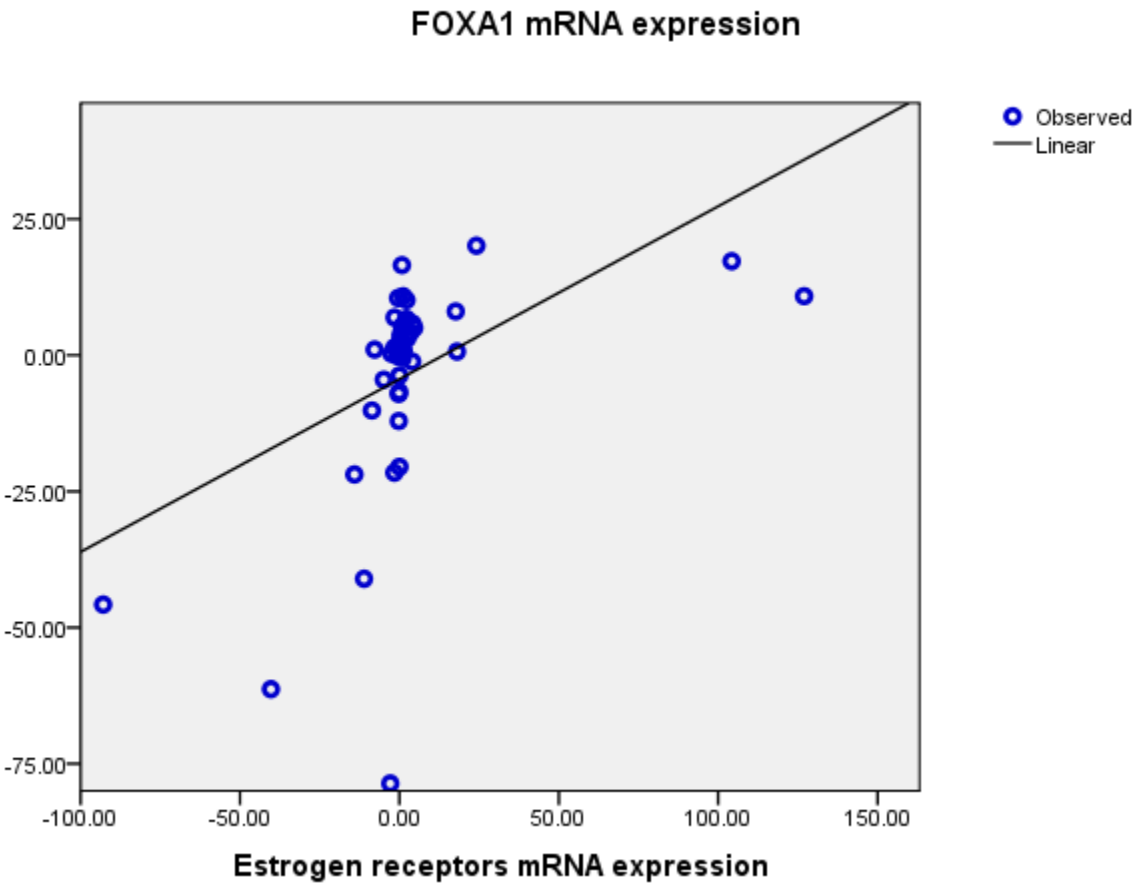

Figure 8 significant positive correction as erythropoietin mRNA expression increase increases the estrogen mRNA expression increase.

Since the three parameters of the same sample weir are going to study the relationship between erythropoietin, FOXA1 and estrogen.

Table 14 ***Correlation's relationship between erythropoietin, FOXA1 and estrogen***

		Erythropoietin mRNA expression	FOXA1 mRNA expression	Estrogen receptors mRNA expression
Erythropoietin mRNA expression	Pearson Correlation	1	.248*	.719**
	Sig. (1-tailed)		.044	.000
	N	48	48	48
FOXA1 mRNA expression	Pearson Correlation	.248*	1	.483**
	Sig. (1-tailed)	.044		.000
	N	48	48	48
Estrogen receptors mRNA expression	Pearson Correlation	.719**	.483**	1
	Sig. (1-tailed)	.000	.000	
	N	48	48	48

**. Correlation is significant at the 0.05 level (1-tailed).*

***. Correlation is significant at the 0.01 level (1-tailed).*

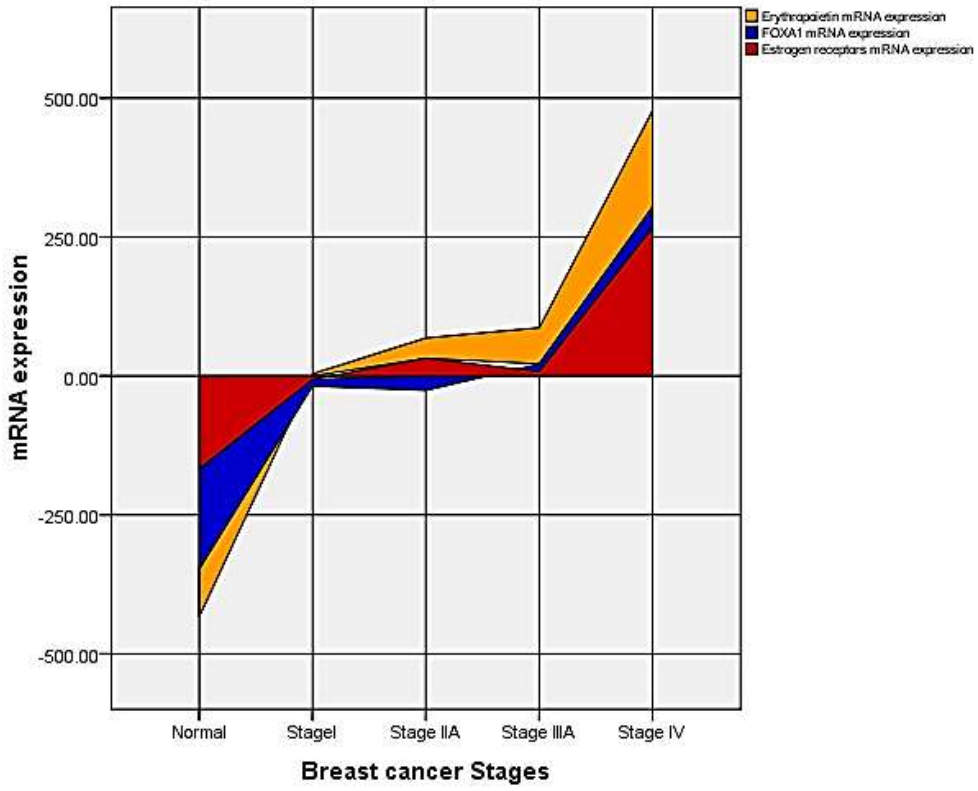

Figure 10: - erythropoietin, FOXA1 and estrogen correlation

Discussion

They are several studies confirm that FOXA1 Erythropoietin hormone and it is receptor as will are cancerogenic.

Positive androgen receptor in prostate cancer and positive estrogen receptors in breast cancer responsiveness to immunotherapy are less compared to different type of cancer but the mechanism is still unknown. FOXA1 overexpression inversely correlated with interferon (IFN) signature and antigen presentation gene expression in PCa and BCa patients. FOXA1 bound STAT2 DNA binding domain and suppressed STAT2 DNA binding activity, IFN signaling gene expression and cancer immune response independently of the transactivation activity of FOXA1 and its mutations detected in prostate and breast cancers. Increased FOXA1 expression promoted cancer immuno- and therapy resistance in mice and PCa and BCa patients. These findings were also valid in bladder cancer expressing high level FOXA1. FOXA1 overexpression could be a prognostic factor to predict therapy resistance and a viable target to sensitize luminal prostate, breast, and bladder cancer to immuno- and chemotherapy.(He et al., 2021)

Forkhead box A1 (FOXA1) pioneer transcription factor (TF) evoking alternative key TFs-mediated lineage-specific transcriptional programs in many endoderm-derived organs. Aberrant FOXA1 augmentation, via genetic alterations, occurs in 10-15% of ER+ primary and metastatic breast cancer (BC). We have recently shown that top levels of FOXA1 (H-FOXA1) induces enhancer and transcriptional reprogramming to promote endocrine-resistant (EndoR) and pro-metastatic phenotypes.(Fu et al., 2021)

Erythropoietin (EPO) plays a range of vital functions within the body. Contrary to original beliefs, its activity is not limited to exerting effects on cells on the erythropoietic pathway. Newly printed results continue to offer data on novel functions of the supermolecule in alternative sorts of tissues, as well as on the important roles contend by EPO in pathological processes. With no doubt, EPO has a significant impact on the biology of carcinoma cells by affecting cells' proliferation, apoptosis, resistance to chemotherapy, as well as expression of assorted sorts of receptors. EPO exerts its direct action on breast cancer stem-like cells by activation of specific signaling pathways liable for protection of the tumor from chemotherapy and fast illness progression. EPO could inhibit chemotherapeutical drug-induced programmed cell death and toxicity.(M.P. Budzik, 2019)

Erythropoietin (EPO) plays role in cancer development and in all probability affects clinical outcomes. A functional polymorphism (rs1617640, G > T) in the promoter region of the EPO increases macromolecule expression. This study investigated the association of EPO rs1617640 with treatment efficacy and severe toxicity in non-small cell lung cancer (NSCLC) patients undergoing platinum-based regimens.(Zheng, Deng, Tang, & Cai, 2021)

So, they are many of studies in different way confirm the role of Erythropoietin FOXA1 are risk factors for development and progression of Breast cancer and cancer in general. In this study we are going to identify the relation between three component Erythropoietin, FOXA1 and estrogen expression in the same sample to make sure the correlation.

They are strong positive correlation between erythropoietin, and FOXA1 and estrogen mRNA gene expression figure 10 table 13.

Conclusion

Erythropoietin hormone and its receptor is cancerogenic in androgen tissue depending, like prostate gland and breast through activation FOXA1 which in turn increase the activity of number of estrogen receptors expression, erythropoietin and FOXA1 correlation is regard as novel approach therapeutic targeting for breast cancer.

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