RESEARCH ARTICLE

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Evaluation of *KISS1* Receptor Gene Expression in Egyptian Female Patients with Breast Cancer

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Abstract

Objective: Breast cancer is the second most common cancer in the world. Many metastasis suppressor genes were identified, including the *KISS1* gene which encodes for a 145 amino acid protein (kisspeptin-145), which undergoes proteolytic cleavage resulting in kisspeptin-14, -13 and -10. All of these proteins can activate KISS1 receptor (KISS1R). The role of KP/KISS1R signaling in breast cancer remains controversial. The present study aimed to measure mRNA gene expression of *KISS1* receptor in healthy and cancerous breast tissue and to evaluate the association of its level with the available molecular subtypes and the traditional clinico-pathological variables. **Methods:** The study was done on 41 operable primary breast cancer patients. Biopsies from both tumor tissue and surrounding healthy mammary tissue were taken from all patients. KISS1R mRNA expression level was measured using a quantitative real time PCR. **Results:** KISS1R mRNA expression was significantly higher in stage III patients compared to stage II patients. At a cut-off value for KISS1R mRNA expression of 1.75, stage II was discriminated from stage III. A significant positive correlation was found between KISS1R mRNA expression and tumor size as well as lymph nodes metastasis. KISS1R mRNA was highly expressed in ER negative cases compared to ER positive ones, and in PR negative cases compared to PR positive ones. There was a statistically significant difference in KISS1R mRNA expression levels and different molecular subtypes being over-expressed in HER2 and triple negative cancer cases. **Conclusion:** This study supports other studies suggesting that KISS1/KISS1R may not be acting as a metastasis suppressor in breast cancer. KISS1R mRNA is over expressed in advanced stages of breast cancer and hence it can be used as a prognostic marker for aggressiveness of breast cancer. Also being over expressed in triple negative patients, KISS1R could represent a promising therapeutic target in triple negative cases.

Keywords: Breast cancer- molecular subtypes- *KISS1R*

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Introduction

Worldwide, breast cancer (BC) is the most common cancer among women (Ferlay et al., 2015). According to the 2014 published data of the Egyptian National Population Based Cancer Registry Program, breast cancer incidence in Lower, Middle, and Upper Egypt accounted for 33.8%, 26.8% and 38.7% respectively of all cancer types in females (Ibrahim et al., 2014).

Metastatic disease remains a significant contributor to morbidity and mortality in patients with breast cancer. An improved molecular and biochemical understanding of the metastatic process is expected to fuel the development of new therapeutic approaches (Tao et al., 2015).

Metastasis suppressors, defined by their abilities to inhibit metastasis without blocking tumor growth, are attractive agents to treat metastasis (Bodenstine and Welch, 2008). Several metastasis suppressor genes were identified, among which *KISS1* gene is of interest (Lee and Welch, 1997). It encodes a protein that when cleaved results in short biologically active peptides collectively referred to as kisspeptins (KPs), (Ohtaki et al., 2001).

Kisspeptins (KPs) are the endogenous ligands for the orphan G-protein coupled receptor 54 (GPR54 or KISS1R) (Muir et al., 2001). The *KISS1R* gene is located on chromosome 19p13.3. It consists of 4322 base pairs and contains 5 exons (Lee et al., 1999). The KISS1 and KISS1R mRNA are expressed at several sites throughout the body including the normal breast, placenta, pituitary gland, pancreas, spinal cord, heart, skeletal muscles, kidney, liver and central nervous system (Kirby et al., 2010; Blake et al., 2017).

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Two signaling pathways are proposed for the KISS1R upon ligand binding; a G- protein dependent pathway and a G-protein independent (β-arrestin dependent) pathway. In the first pathway, KISS1R activates the primary effector Phospholipase-C (PLC), resulting in the formation of 1,4,5 inositol trisphosphate and diacylglycerol, with the resultant mobilization of calcium and activation of protein kinase-C as well as extracellular signal regulated kinases -1 and -2 (ERK1/2) (Cvetkovic et al., 2013). In the alternate pathway, KISS1R can cause activation of ERK1/2 via arrestin-β2. In tumour cells, KISS1R can inhibit ERK1/2 via arrestin-β1, which can subsequently lead to inhibition of nuclear factor κB (NFκB), likely via accumulation of cytoplasmic inhibitory component (IκB), with the resultant reduction of NFκB binding to the matrix metalloproteinase (MMP)-9 promoter and the subsequent decrease of MMP-9 expression (Yan et al., 2001).

Furthermore, KISS1R activity has been shown to increase production of tissue inhibitor of matrix metalloprotease-1, inhibit cell migration, and increase activity of focal adhesion kinase (FAK), leading to formation of excessive focal adhesions and stress fibres, as well as inhibition of cell proliferation, invasion, chemotaxis and metastasis (Kotani et al., 2001).

Although *KISS1* gene is commonly classified as a metastasis suppressor gene and reduction in *KISS1* and/or KISS1R expression correlates with poor patient prognosis in ovarian, oesophygeal, gastric and breast cancers, (Cvetkovic et al., 2013) yet another study has shown that the over expression of KISS1 and KISS1R correlates with breast tumor progression and poor patient prognosis (Martin et al., 2005). Moreover, KISS1 and KISS1R mRNA expression have been found to be elevated in cancerous compared to normal mammary tissue (Marot et al., 2007).

Classical immunohistochemistry (IHC) markers such as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2), together with traditional clinico -pathological variables including tumor size, tumor grade and nodal involvement, are conventionally used for patient prognosis and management (Vallejos et al., 2010) These immunohistochemistry markers are also used for molecular subtyping of breast cancer which can divided into four main molecular subtypes (Luminal A (ER+ / PR+ / HER2-), Luminal B (ER+ / PR+ / HER2-) or (ER+ / PR+ / HER2+), Triple negative (ER- / PR- / HER2-) and HER2 over expression type (ER- / PR- / HER2+) (Spitale et al., 2009).

Marot et al., (2007), studied human breast cancer tumors and hypothesized that KISS1 and KISS1R were estrogen regulated genes.

Current reports concerning the mRNA expression of KISS1R in breast cancer are few; hence it was noteworthy to study its mRNA level in cancerous and normal breast tissue to clarify its potential roles in breast cancer.

Subjects and methods

The study was conducted on forty one (41) operable primary breast cancer patients, from whom biopsy from both tumor tissue and surrounding normal healthy mammary tissue was taken after leaving a safety margin. Females receiving preoperative radio- or chemotherapy as well as those suffering from malignancies other than breast cancer were excluded.

Full clinical examination and radiological investigations including mammogram, chest X-ray and ultrasonography of the abdomen were done for all cases. Histopathologically confirmed diagnosis of breast cancer including staging was also done. Immunohistochemical determination of estrogen receptor, progesterone receptor and human epidermal growth factor2 receptor (HER2) were carried out in paraffin embedded breast tissue sections. All patients were selected from patients admitted to the Experimental and Clinical Surgery unit of Medical Research institute, Alexandria University Hospital.

Written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. The study was approved by the Ethics Review Board of Medical Research institute, Alexandria University.

Fresh tissue sections were collected from both tumor tissue and normal mammary tissue and were immediately immersed in a ribonucleic acid 'RNA later' stabilizing solution, then stored at -20^{o}C till time of extraction. Total RNA was extracted using PureLink® RNA Mini Kit (Life Technologies, Carlsbad, CA, USA). Purity and concentration of RNA were assessed using Nanodrop 2000/2000c (Thermo Fisher Scientific, Waltham, MA, USA). Purified RNA was stored at −80°C.

Then complementary deoxy ribonucleic acids (cDNA) was synthesized using High Capacity cDNA .Reverse Transcription Kit (Archive, Applied Biosystems, USA,). Each reaction comprised approximately 10 µg RNA extract, 2 µl of reverse transciptase Buffer, 0.8 µl of deoxy nucleotide triphosphate (dNTP), 1 µl of reverse transciptase, 1µl RNase Inhibitor, 2 µl RT Random Primers, then the total volume was completed to 20 µl using nuclease-free water. The thermal cycle was programmed at 10 min hold at temperature 25°C, 120 min hold at temperature 37°C, 5 min hold at temperature 85°C, then lowering the temperature to 4°C and stopping the run. Following reverse transcription, cDNA was stored at -20°C to be used in real time quantitative polymerase chain reaction experiments (RT-qPCR).

Real-time PCR was performed on cDNA on Applied Biosystems Step-one Real-time using Thermo Scientific Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific, Cat. No. K0251), and specific primers for KISS1R and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control, Table1). Each reaction contained 12.5 µl Maxima SYBR Green qPCR Master Mix (2X), 1 µl forward Primer , 1 µl reverse primer , 0.1 µl ROX Solution, 7.4 µl nuclease free water and 3 µl cDNA. Samples were assayed in duplicates. A no template control (NTC) was performed in each assay. RT-qPCR was programmed as follows: an initial cycle of 95°C, 10 minutes; followed by 3 –step cycling: (40 cycles) Denaturation 95°C, 15 seconds ; Annealing 53°C forKISS1R , and 65°C for *GAPDH* gene for 30 seconds and finally Extension step 72°C for 30 seconds. Melting curve was performed to verify specificity and identity of

the PCR products.

KISS1R expression level was calculated using the comparative cycle threshold (CT) method ($2^{-\Delta\Delta CT}$). Relative quantification is the most common method used to detect RNA expression differences between two samples. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous control in this study (Rao et al., 2013). Results were analyzed using The StepOne™ Software.

Results

The present study included 41 BC female patients with a median age of 53 years (28 – 89 years). According to TNM-staging system, 24 cases (58.5%) were stage II and 17 cases (41.5%) were stage III. LN metastasis was detected in 27 patients (65.9%) and absent in 14 patients (34.1%), Table 2. Other demographic and clinical data, histological grading, tumor size as well as hormone receptor status and molecular subtypes of BC are shown in Table 2.

As regards KISS1R mRNA expression level, The KISS1R mRNA was over expressed in 24 cases (58.5%) and under expressed in 17 cases (41.5%), Table 2. Moreover, a highly significant difference in KISS1R mRNA expression was noted in stage III patients (median 3.21, Min=1.17, Max=4.75) compared to stage II patients (median 0.69, Min=0.05, Max=2.08) ($p < 0.001$), Table 3, Figure 1.

Table 1. The Primer Sequences for KISS1R (Marot et al., 2007) and GAPDH (Singh et al., 2016)

Primer name	5'-3' sequence
KISS1R (forward)	CGACTTCATGTGCAAGTTCGTC
KISS1R (reverse)	CACACTCATGGCGGTCAGAG
GAPDH (forward)	AAATCAAGTGGGGCGATGCTG
GAPDH (reverse)	GCAGAGATGATGACCCTTTTG

Table 2. Distribution of Breast Cancer Patients According to age, KISS1R mRNA Expression Levels and the Histopathological Findings

Variable	Number (%)
Age	Median& range:53 years (28 – 89 years)
KISS1R mRNA expression	
Over expressed	24 (58.5)
Under expressed	17 (41.5)
Molecular subtype	
Luminal A	13 (31.7)
Luminal B	14 (34.1)
HER2 over expression	9 (22.0)
Triple negative	5 (12.2)
Grade	
Grade 2	35 (85.4)
Grade 3	6 (14.6)
Stage	
Stage 2	24 (58.5)
Stage3	17 (41.5)
Tumor size	
≤2 cm	5 (12.2)
>2 cm	36 (87.8)
Lymph node metastasis	
Negative	14 (34.1)
Positive	27(65.9)
Vascular invasion	
Negative	3 (7.3)
Positive	38 (92.7)
Capsular and extra nodal fat deposits	
Negative	14 (34.1)
Positive	27 (65.9)

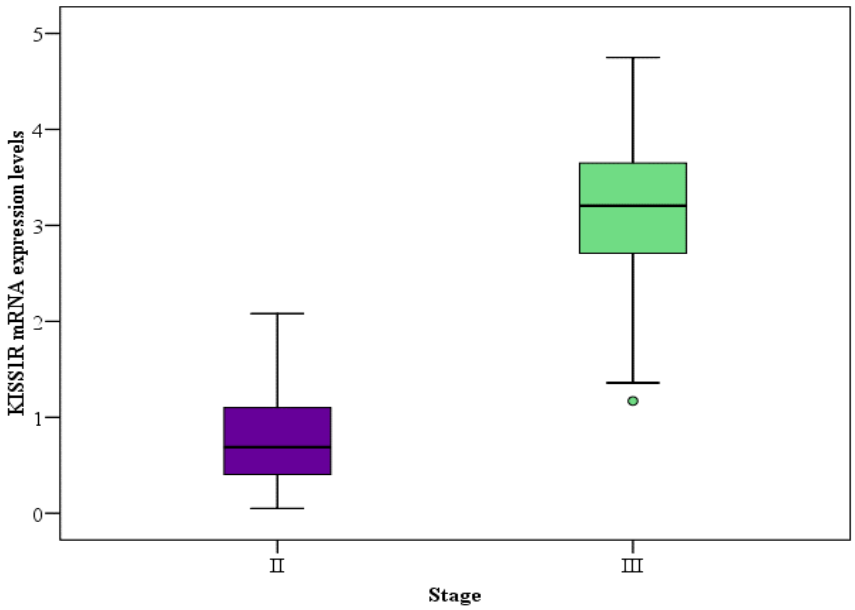

Figure 1. Box Plot Showing the Relation between KISS1R mRNA Expression Levels and Tumor Stage (n = 41) (o) Represents the Outlier Observation

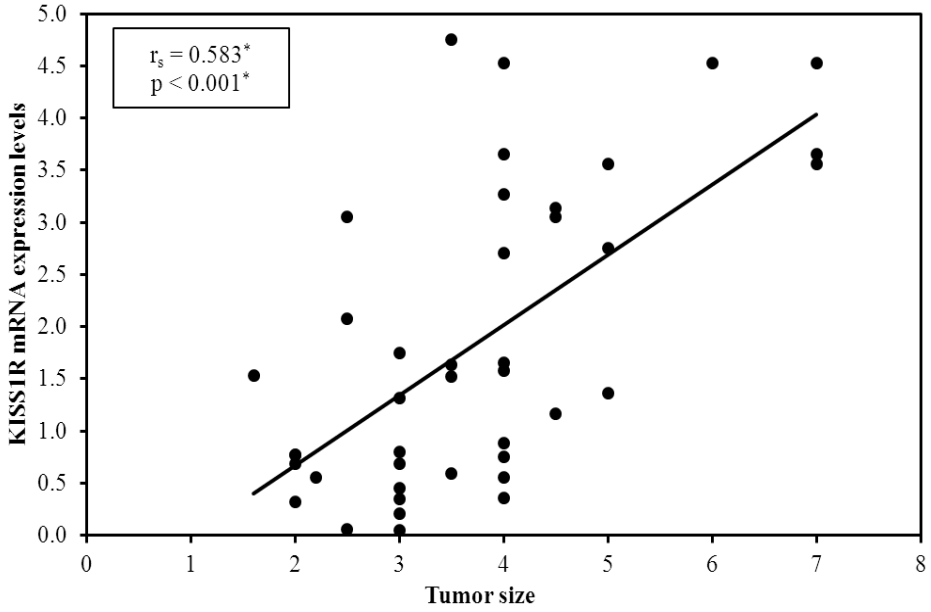

Figure 2. Correlation between KISS1R mRNA Expression Levels and Tumor Size (n = 41)

As regards hormonal receptors, there was a statistically significant difference in KISS1R mRNA expression between ER positive and negative patients being highly expressed in ER negative patients (median value = 2.75 [0.88 – 4.53]) compared to ER positive patients (median value=0.77 [0.05 – 4.75]) (p=0.005). Furthermore, there was a highly significant difference in KISS1R mRNA expression between PR positive and negative patients being highly expressed in PR negative patients (median value=2.75 [0.88 – 4.53]) than PR positive patients (median value=0.77 [0.05 – 4.75]) (p=0.005), Table (3).

A statistically significant difference was noted in KISS1R mRNA expression among the different molecular subtypes, being over expressed in HER2 (median value=3.05 [1.52 – 4.75]) and triple negative (median value=2.75 [1.32 – 4.53]) subtypes and under expressed in luminal A (median value=0.77 [0.21 – 3.65]) and luminal B (median value = 0.78 [0.05 – 4.53]) subtypes (p=0.005), Table 3.

There was no statistically significant difference in KISS1R mRNA expression among the remaining variables (age, tumor grade, tumor size, lymph node metastasis, vascular invasion, capsular and extra nodal fat deposits as well as HER2 receptors), Table 3. A significant positive correlation was noted between KISS1R mRNA expression and both tumor size (r=-0.583*, p<0.001*) and Lymph nodes metastasis (r=0.370*, p=0.017*), Figures 2 and 3. While, no significant correlation was found between KISS1R mRNA expression and age of the patients.

At a cut-off value for KISS1R mRNA expression

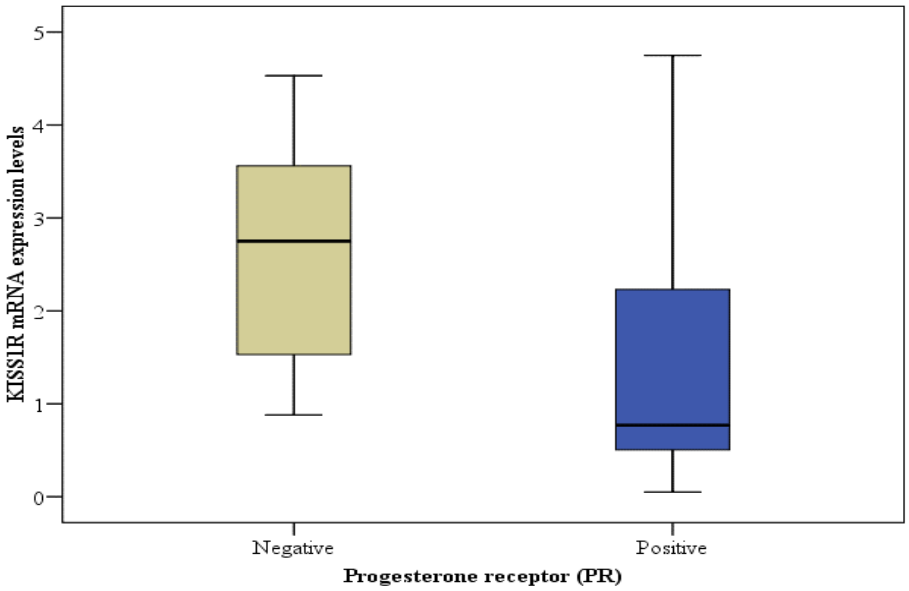

Figure 3. Box Plot Showing the Relation between KISS1R mRNA Expression Levels and Progesterone Receptor (PR) (n = 41)

of 1.75, stage II was discriminated from stage III with a diagnostic sensitivity of 77.78 % and a specificity of 95.65 %, with an overall test accuracy of 87.80%, Table 4 and Figure 4.

Discussion

Breast cancer is the second most common cancer in the world and by far the most frequent cancer among women (Bray et al., 2018). The advances in molecular biology aid in better understanding of breast cancer, enabling the design of smarter therapeutics that are able to target cancer and respond to its microenvironment efficiently (Nounou et al., 2015).

The *KISS1* gene encodes for a 145 amino acid protein known as kisspeptin-145, which undergoes a series of proteolytic cleavage resulting in kisspeptin-14, -13 and -10. All of these peptides can activate KISS1 receptor

Table 3. Relation between KISS1R mRNA Expression and Different Variables (n = 41)

	No	KISS1R mRNA expression		Test of sig.	p
		Min. – Max.	Median		
Age					
<50	11	0.05 – 4.75	2.75	U=	0.391
≥50	30	0.06 – 4.53	1.44	135	
Stage					
II	23	0.05 – 2.08	0.69	U=	<0.001*
III	18	1.17 – 4.75	3.21	19.0*	
Grade					
II	35	0.05 – 4.75	1.53	U= 92.0	0.653
III	6	0.21 – 4.53	0.97		
Tumor size					
≤ 2 cm	5	0.32 – 1.53	0.77	U=	0.128
> 2 cm	36	0.05 – 4.75	1.61	51.5	
Lymph node metastasis					
Negative	14	0.05 – 4.53	1.05	U=	0.143
Positive	27	0.21 – 4.75	1.58	135.5	
Vascular invasion					
Negative	3	0.35 – 4.53	0.36	U=	0.551
Positive	38	0.05 – 4.75	1.53	44	
Capsular and extra nodal fat deposits					
Positive	14	0.05 – 4.53	0.79	U= 124.0	0.076
Negative	27	0.21 – 4.75	1.58		
Estrogen receptor (ER)					
Negative	13	0.88 – 4.53	2.75	U= 83.5*	0.005*
Positive	28	0.05 – 4.75	0.77		
Progesterone receptor (PR)					
Negative	13	0.88 – 4.53	2.75	U= 83.5*	0.005*
Positive	28	0.05 – 4.75	0.77		
HER2					
Negative	17	0.21 – 4.75	1.75	U= 186.5	0.643
Positive	24	0.05 – 4.53	1.27		
Molecular subtype					
HER2 over expression	9	1.52 – 4.75	3.05	H= 12.815*	0.005*
Luminal A	13	0.21 – 3.65	0.77		
Luminal B	14	0.05 – 4.53	0.78		
Triple negative	5	1.32 – 4.53	2.75		
Sig. bet. grps		p1=0.003*,p2=0.006*,p3=0.807,p4=0.793,p5=0.031*,p6=0.047*			

U, Mann Whitney test; H, H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test); p, p value for association between different categories; p1, p value for comparing between HER2 over expression and Luminal A; p2, p value for comparing between HER2 over expression and Luminal B; p3, p value for comparing between HER2 over expression and Triple negative; p4, p value for comparing between Luminal A and Luminal B; p5, p value for comparing between Luminal A and Triple negative; p6, p value for comparing between Luminal B and Triple negative; *, Statistically significant at p ≤ 0.05

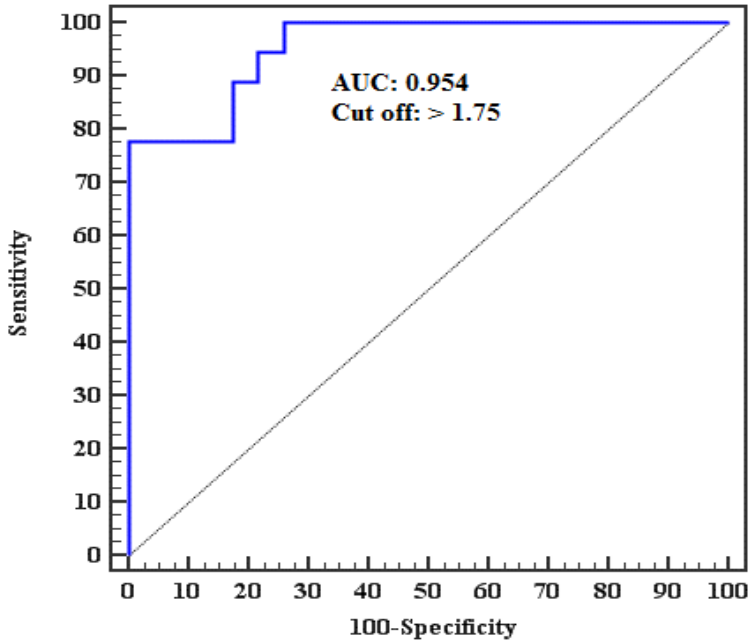

Figure 4. ROC Curve for KISS1R mRNA Expression to Discriminate Stage III from Stage II

Table 4. Agreement (Sensitivity, Specificity) for KISS1R mRNA Expression to Discriminate Stage III from Stage II

	AUC	p	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
			LL	UL						
KISS1R mRNA expression levels	0.954	<0.001*	0.898	1	>1.75	77.78	95.65	93.3	84.6	87.8

AUC, Area Under a Curve; p value, Probability value; CI, Confidence Intervals; NPV, Negative predictive value; PPV, Positive predictive value ; *, Statistically significant at $p \leq 0.05$; #, Cut off was choosed according to Youden index

(KISS1R) (Pinilla et al., 2012).

The decrease in KISS1 and/or KISS1R mRNA expression has been shown to be associated with poor clinical prognosis in some cancer patients. So, the expression of KISS1 and/or KISS1R could be powerful prognostic markers in clinical settings (Lee et al., 1996; Shirasaki et al., 2001; Dhar et al., 2004; Prentice et al., 2007; Canbay et al., 2012).

The role of KP/KISS1R signaling in breast cancer remains controversial and hence the present study was carried out to measure the *mRNA* gene expression of KISS1 receptor in healthy and cancerous breast tissue of patients with breast cancer, and to evaluate the association of its level with the available molecular subtypes and the traditional clinico-pathological variables.

The study was conducted on forty one operable primary breast cancer patients, from whom biopsy from both tumor tissue and surrounding normal healthy mammary tissue was taken after leaving a safety margin.

Full clinical examination, radiological investigations, histopathologically confirmed diagnosis of breast cancer including staging and Immunohistochemical determination of estrogen receptor, progesterone receptor and human epidermal growth factor2 receptor (HER2) were done. Relative quantification of KISS1R mRNA expression level was done on fresh tissue sections of the biopsy using a quantitative real time PCR (RT-qPCR).

In the present study, a highly significant KISS1R mRNA expression was noted in cases with BC stage III compared to those with BC stage II. This finding was in agreement with several studies. One study done by Martin et al. (2005) revealed that levels of KISS-1 mRNA expression showed a relative increase in stage III patients, yet not reaching the level of statistical significance. They observed that KISS-1 mRNA expression was increased with higher grade of tumor; however the differences were not statistically significant. There was a little difference in KISS1R expression among different tumor grades. This study also evaluated how the introduction of the *KISS1* gene into human breast cancer cells increased their invasive phenotype in in-vitro assays (Martin et al., 2005).

Another study by Marot et al., (2007) reported that KISS1 mRNA and KISS1R mRNA were highly expressed in invasive breast tumor (Marot et al., 2007) The studies of (Martin et al., 2005) and (Marot et al., 2007) as well as others (Goertzen et al., 2016) demonstrated that KISS1 / KISS1R signaling may not function as a metastasis suppressor in breast cancer, but the underlying mechanisms were unknown. Jarząbek et al. (2012) found that KISS1R expression was higher in the moderately differentiated breast tumor (G2) compared to the poorly differentiated high grade (G3) tumor (Jarząbek et al., 2012).

The fact the KISS1 / KISS1R signaling is promigratory and pro invasive in human breast cancer cells was explained in a study done by Goertzen et al. (2016) whom demonstrated that KISS1R signaling induces invadopodia formation and activation of key invadopodia proteins, cortactin, cofilin and membrane type I matrix

metalloproteases (MT1-MMP). Moreover, KISS1R stimulated invadopodia formation via a new pathway involving a β-arrestin2 and ERK1/2-dependent mechanisms. Such findings suggest that targeting the KISS1R signaling axis might be a promising strategy to inhibit invasiveness and metastasis (Goertzen et al., 2016).

In the present work, the ROC curve analysis done for KISS1R mRNA expression to discriminate stage II from stage III revealed an AUC of 0.954, where a cut-off value of 1.75 was generated to discriminate stage II from stage III with a diagnostic sensitivity of 77.78 %, specificity of 95.65 %, positive (PPV) and negative (NPV) predictive values of 93.3 % and 84.6 % respectively, with an overall test accuracy of 87.80%.

Correlation studies done in our work revealed a significant positive correlation between KISS1R mRNA expression and lymph node metastasis (r = 0.370*, p = 0.017*). (Table 4.5) Martin et al. (2005) demonstrated that KISS1 mRNA expression was significantly higher in lymph node positive breast tumors compared to lymph node negative tumors as well as KISS1R mRNA expression that was also reported relatively higher in lymph node positive tumors compared to lymph node negative tumors, yet did not reach the level of statistical significance.

On the other hand, several studies suggested that a loss of KISS1 may lead to the formation of distant metastases, since there was a down-regulation of KISS1 expression in the metastases compared to primary tumors (Stark et al., 2005; Kostadima et al., 2007; Mooez et al., 2011; Jarząbek et al., 2012; Ulasov et al., 2012; Xie et al., 2012). Some studies reported that KISS1 mRNA and protein expression were absent in node positive breast cancer, and found a significant negative correlation with axillary lymph node involvement (Kostadima et al., 2007; Mooez et al., 2011; Xie et al., 2012). KISS1 mRNA and protein expression were also found to be significantly higher in primary breast cancer compared to breast tumors that metastasized to the brain (Stark et al., 2005; Ulasov et al., 2012). Such studies support the anti-metastatic role of KISS1.

Tumor size is an important factor influencing the lymph node involvement in breast cancer (Xie et al., 2012). Wada et al., (2006) found that tumor size lager than 2 cm was considered a predictor of tumor involvement in remaining axillary lymph nodes of breast cancer patients with lymph node involvement (Wada et al., 2006). In that context, our study showed a significant positive relation between KISS1R mRNA expression level and tumor size (r=-0.583*, p <0.001*) (Tables 4). In a study done by Jarząbek et al. (2012) on KISS1, a statistically negative correlation was found between the tumor size and mRNA expression level of KISS1 (Jarząbek et al., 2012).

As regards the immunohistochemical staining of receptors, the present study demonstrated a significantly higher KISS1R mRNA expression in ER negative patients (median 2.75, Min=0.88, Max=4.53) compared to ER positive patients (median 0.77, Min=0.05, Max=4.75) (p=0.005). These results were in accordance with a study done by Jarzabek et al., (2015) whom observed that KISS1R expression was higher in ERα negative cases compared to ERα positive cases in patients with lymph node involvement. The expression and function of both KPs and KISS1R are modulated by estrogen. Estradiol (E2) acts through estrogen receptor (ER)-α which is an important prognostic indicator in breast cancer for the response to endocrine therapy (Stingl, 2011). KISS1 and KISS1R expressions were found to be negatively regulated by E2 via ERα (Izadi et al., 2012; Shi et al., 2012).

In addition, the present study demonstrated that KISS1R mRNA expression was significantly higher in PR negative patients (median=2.75 [0.88 – 4.53]) compared to PR positive patients (median=0.77 [0.05 – 4.75]) (p=0.005) (Table 4). Jarzabek et al., (2015) reported a positive correlation between KISS1R mRNA expression and the PR status.

Despite the lack of a significant relation between KISS1R mRNA expression and the HER2/neu status in our study, yet Jarząbek et al., (2012) reported that KISS1R mRNA expression negatively correlated with the HER-2/neu status where it was significantly higher in the HER-2/neu negative tumors compared to the HER-2/neu positive tumors (Jarząbek et al., 2012). In that context, Ulasov et al., (2012) found no significant correlation between KISS1 and HER2 or ER stains, but they found a significant positive correlation between KISS1 expression and PR status.

Marot et al., (2007) reported that KISS1 mRNA level was significantly lower in ER positive breast tumors when compared with the ER negative breast tumors, while KISS1R mRNA expression was slightly (but not significantly) higher in ER positive breast tumors than ER negative tumors.

The present study found that there was a statistically significant difference in KISS1R mRNA expression and different molecular subtypes being over-expressed in HER2 subtype and triple negative (TNBC) subtype while under expressed in luminal A and luminal B subtypes (p=0.005). In accordance with that finding, Blake et al., (2017) found that KISS1 and KISS1R mRNA expression level and KISS1R protein were up-regulated in TNBC tumors, compared to normal breast tissue. They also demonstrated that KISS1R signaling promotes drug resistance by increasing the expression of efflux drug transporter, breast cancer resistance protein (BCRP) and by inducing the activity and transcription of the receptor tyrosine kinase. They provided evidence that KISS1R is a key regulator of drug resistance. Thus, KISS1R represents a potentially novel therapeutic target to regain drug sensitivity in TNBC patients (Blake et al., 2017).

In conclusion, the results of this study support the notion that KISS1R mRNA expression may not be functioning as a metastasis suppressor in breast cancer cases. On the contrary, KISS1R is over expressed in advanced stages of breast cancer and hence it can be used as a prognostic marker for aggressiveness of cancer. Being over-expressed in TNBC cases, KISS1R could represent a promising therapeutic target in triple negative cases. Nevertheless, further studies on larger number of triple negative breast cancer cases are highly recommended to document such a finding.

Author Contribution Statement

All the authors contributed equally to this work

Acknowledgments

We would like to thank all patients for their contribution to this work.

Ethics approval

The study was approved by the Ethics Review Board of Medical Research institute, Alexandria University.

Availability of data and material

The data analyzed during this study are available from the corresponding author on reasonable request.

Conflict of interest

We clarify that no conflict of interest is related to this publication.

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