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Evaluation the Gene Expression of *P27* and *SKP2* Genes in Iraqi Women Patients with Breast Cancer

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Abstract

The current experiment have been conducted to evaluate the suppression activity of P27 and SKP2 genes by using qRT- PCR, Blood samples are taken from 60 patients women with breast cancer to represent patients group and 40 women's without breast cancer to represent control group, the ages of both groups ranged from 30 to more than 50 years in the Al.Diwaniyah General Teaching Hospital in AL-Qadisiyah province. Also ROC curve analysis is used to demonstrate the accuracy and validity of previous parameters in detecting women with breast cancer and to estimate the sensitivity and specificity.The obtained results showed that the control group has showed significantly ($p<0.01$) effect on gene expression for P27 (10.882 fold change)comparison with patients groups (4.496 fold change), while the results of this study have explained that the gene expression of SKP2 for patients group have inquired high significant ($p<0.01$) superiority which is 13.943 fold change compared to 3.494 fold change for control group . The age groups have no significant effect on gene expression for SKP2 and P27 genes.Analysis results of ROC curve for P27 gene expression explain it is a good indicator to detecte the actually patients with breast cancer ,where as the sensitivity and specificity is 81.7 and 22.5% respectively and the cut-off for P27 is less than 6.52 fold change. while the gene expression of SKP2 is non- efficient to detecte patients with breast cancer, where as the cut-off is more than 4.486 fold change ,the sensitivity and the specificity is 63.3 and 20%respectively.

Keywords
breast cancer, p27and skp2 genes.

Breast cancer is one of the most prevalent cancers affecting women. with a mortality rate of more than one million per year throughout the world.In 2020, there were 2.3 million women diagnosed with breast cancer and 685, 000 deaths globally. The most common cancer in the world as of the end of 2020 was breast cancer, which had been diagnosed in 7.8 million women in the previous five years (WHO,2020). In women, breast cancer accounted for almost 24.5% of cases, placing it first for incidence and mortality in the vast majority of countries worldwide (Sung *et*

al.,2020). and 4.4 million cases are predicted in 2070 (Soerjomataram and Bray,2021). Despite being frequently thought of as a disease that only affects women, breast cancer (BC) can also affect men. Male Breast Cancer (MaBC) is uncommon (Makdissi *et al.*,2022). The development of this condition is influenced by both genetic and environmental factors (Benvidi *et al.*, 2015). This condition ranks as the second leading cause of cancer-related death in women between the ages of 45 and 55 years (Jemal *et al.*,2009), and is the second most common reason for cancer-related death. Breast cancer affects almost one in eight women, and the majority of the time it requires complete tissue removal, chemotherapy, radiotherapy, and hormone therapy (Heravi-Karimovi *et al.*,2006).Age, menarche, menopause, parity, breastfeeding, use of exogenous hormones or oral contraceptives, obesity, lack of exercise, diet, smoking, alcohol consumption, and family history of breast cancer or other cancers are just a few of the breast cancer-associated risk factors that have been suggested to play a role in the development of breast cancer. Breast cancer is regarded as clinically heterogeneous and Various pathological conditions and clinical behaviors are present in complex disease. Therefore, it is generally accepted that the accumulation of genetic abnormalities contributes to the development of breast cancer that is becoming more and more invasive. This heterogeneity is strictly linked to individuals and tumors of genetic variability (Cavallaro *et al.*, 2021).There are some genes that control cell growth, suppress their carcinogenesis, and regulate their work, called tumor suppressor genes. Important genes called tumor suppressors control a variety of biological processes inside the genome. These genes can be generally categorized according to how they contribute to cell growth and cycle development, cell proliferation, DNA repair processes, and other essential cellular signaling activities like the induction of apoptosis. Dysregulated cell proliferation, a well-known mechanism for the formation of malignancies, is more likely in the absence of functional tumor suppressor genes, An example of such genes (*BRCA 1 and BRCA 2, P21,P27,SKP2,P53,P57*,etc) (Joyce *et al.*,2018).Negative regulators of the cell cycle *P27* and *Skp2. P27* and S-phase kinase-associated protein 2 (*SKP2*) are regarded as tumor suppressor genes because a loss of their function can lead to malignant behavior. *P27*, which was first discovered in 1993, is a member of the Cip/Kip family of cyclin-dependent protein kinase inhibitors (CKIs), along with *P21* and *P57*.49 CKIs slow the cell cycle's progression; *P27* is able to bind to a variety of distinct cyclin/CDK complexes and inhibit their activity, typically causing the cell to arrest in the G1 phase. It has been established that *P27* has distinct binding sites for cyclin and CDK2, and that attachment to this complex alters the catalytic complex's conformation (Russo *et al.*,1996).S-phase kinase-associated proteins(*SKP2*) that is necessary for the ubiquitination and subsequent degradation of *P27*, which causes cells to enter the S-phase.(Bast *et al.*,2001). S-phase kinase-associated protein 2 (*SKP*), an F-box protein, is known to play a significant role in oncogenesis and tumor progression by focusing on substrates for ubiquitination and degradation (Cai *et al*,.2020). S-phase kinase-associated protein 2 (SKP2) is an oncogene and cell cycle regulator that catalyzes the ubiquitination of phosphorylated cell cycle regulator proteins (Lin *et al*,.2019). Most types of human tumors, including breast cancer, have been documented to overexpress Skp2. Skp2 is also highly expressed in human cancer patients, and this is associated with poor survival (Wang *et al*,.2012)Additionally, Skp2 promotes breast cancer metastasis, drug resistance, migratory and invasive activity, and cell proliferation (Cai *et al*,.2020). Low *P27* and high *SKP2* levels in breast cancers are more frequently linked to an ER-

negative phenotype, a high histological grade, and a poor prognosis (Traub *et al.*,2006).

This work was aimed to monitor the role of the regulatory genes SKP2 and P27 in patient of breast cancer.

Materials and Methods

Study group

This study included 60 female patients with the breast cancer of AL-Qadisiyah province who attended to oncology unit of AL-Diwaniyah teaching hospital. Their age range from (30–50⩽) years and over The study also included apparently healthy control 40 individual.

Blood samples

Two ml of blood from 60 patients 40 healthy women were collected, kept two ml in a plastic tube containing an anticoagulant (EDTA), and transported to the laboratory for molecular tests.

Inclusion Criteria

The included criteria were all women patients with breast cancer.

Exclusion Criteria

Excluded criteria all men infected with breast cancer women who was suffering from other chronic disease and cancers.

Ethical approval

This work was done meeting all the formal regulations of AL- Qadisiyah university and the ministry of health of iraq also all patients were informed and formal acceptance were obtained.

Molecular analysis

(A)-Materials used in the study: The real-time measurement of polymerase chain reaction was used for quantitative reverse transcription from Bio-Rad/ USA using the following primers for P27 and Skp2 genes, The primers for Skp2 and P27 target genes and Gapdh housekeeping gene were designed by using NCBI-Gene Bank data base and Primer 3 design online**.** These primers were provided by (Bioneer company, Korea) as following table:

Table (1): show Primers that used in qRT-PCR reaction for detection gene expression

Primer		Sequence (5'-3')	Product Size	NCBI Reference Sequence
P27 gene	F	AGGGGCGCTTTGTTTTGTTC	93bp	NM_004064.5
	R	ACACTCGCACGTTTGACATC		
Skp2	F	ACACTGCAAAAGCCCAGTTG	143bp	NM_001243120.2
	R	TGCAGAATGAAGGCAAAGGG		
Gapdh gene	F	AATTCCATGGCACCGTCAAG	104bp	NM_001289745.3
	R	ATCGCCCCACTTGATTTTGG		

(B) -Examination of polymerase chain reaction for real-time reverse transcription (QRT-PCR)

The polymerase chain reaction was investigated for real-time quantitative reverse cloning to measure the quantitative levels of mRNA, which was transcripted from the woman with breast cancer to denote the amount of gene expression for p27 and skp2 genes, also GaPdH gene was used as a Standard Housekeeping gene to calculate gene expression. This examination was carried out according to the following steps:

1 - Extraction of total RNA : Total RNA were extracted from blood samples by using (TRIzol® reagent kit) and done according to company instructions, 250µl blood sample was homogenized by added 750 µl of TRIzol® reagent and 200µl chloroform was added to each tube and shaken vigorously for 15 seconds.The mixture was incubated on ice for 5 minutes. Then centrifuged at 12000 rpm, 4C°, for 15 minutes, Supernatant was transferred into a new Eppendorf tube, and 500µl isopropanol was added. Then, mixture mixed by inverting the tube 4-5 times and incubated at 4C° for 10 minutes. Then, centrifuged at 12,000 rpm , 4C° for 10 minutes.Supernatant was discarded, and 1ml 80%

Ethanol was added and mixed by vortex again. Then, centrifuge at 12000 rpm, 4C° for 5 minutes.The supernatant was discarded and the RNA pellet was left to air to dry.100µl Free nuclease water was added to each sample to dissolve the RNA pellet, Then, the extracted RNA sample was kept at -20.

2. Estimation of extracted total RNA yield

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 .

3- Treatment with enzyme DNase1 : The extracted RNA were 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.

4. cDNA synthesis: 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.

5. Quantitative Real-Time PCR (qRT-PCR): The quantitative Real-Time PCR used in quantification of target gene Skp2 and P27 target gene expression analysis that normalized by housekeeping gene (GAPDH) in patient and healthy blood samples by using Real-Time PCR technique and this method was carried out according to method described by *(Habeeb,2022)* and include the following steps:

1. qPCR master mix preparation

qPCR master mix was prepared by using GoTaq® qPCR Master Mix kit based on SYBER green dye detection of target and housekeeping genes amplification in Real-Time PCR system and include the following table:

Table (2)show qPCR master mix preparation

volume	qPCR master mix
5µL	cDNA template (100ng)
1 µL	Forward primer(10pmol)
1 µL	Reverse primer (10pmol)
10 µL	qPCR Master Mix
3 µL	DEPC water
20 µL	Total

After that, these qPCR master mix component that mentioned above placed in qPCR plate strip tubes and mixed by Exispin vortex centrifuge for 3 minutes, than placed in Miniopticon Real-Time PCR system.

2- qPCR Thermocycler conditions

After that, the qPCR plate was loaded and the following thermocycler protocol in the following table:

Table (3) show qPCR Thermocycler conditions

Repeat cycle	Time	Temperature	qPCR step
1	5min	95 °C	Initial Denaturation
45	20 sec	95 °C	Denaturation
	30 sec	60 °C	Annealing\Extention Detection(scan)

6- Real-Time PCR analysis of data analysis

The results of this study were analyzed by using factorial analysis treatment groups with two levels (patients group and control group)and the second factor was age groups with three levels (30-40,40-50 and 50year and over),general linear method was used according to spss (2013).

Receiver operating characteristic (ROC) curve was used to estimate true positive cases and true negative cases and to calculate the sensitivity and specificity also to know the accuracy of used test ,indetection the patients with breast cancer (zweignd and campbell,1993).

Results and Discussion

A- Prevelance of breast cancer in AL-Qadisyah provience

All data of female patients who attended to the hospital of ages were the number of the category

was from 30-40 years (9) and the percentage was (15%) ,and the number of the category 40-50 years was (15) and the percentage 25% ,The number of the category 50 years and over was (36) and the percentage 60%. as showen in figure(1).

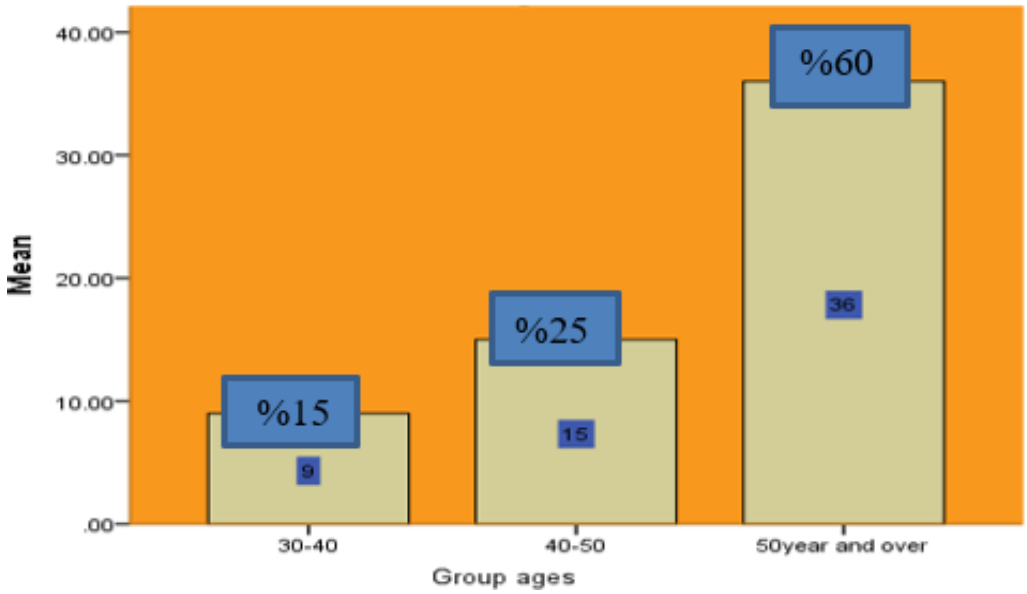

Figure (1) Distribution of patients with breast cancer according to age

B- *P27* gene expression

Table (4) illustrates the the study groups show a significant change ($p<0.01$) , the patients group gene expression was 4.496 in comparison to control; group which was 10.882 .

Table(4)The means ± SD of gene expression of P27

Study group	Age groups	Mean	±SD	Number
Patient group	30-40	5.590	4.102	9
	40-50	3.611	2.443	15
	50 year and over	4.592	4.215	36
	Total	4.496 A	3.824	60
Control group	30-40	10.584	5.174	22
	40-50	10.626	5.680	12
	50 year and over	12.485	6.613	6
	Total	10.882B	5.439	40
Total	30-40	9.134 a	5.342	31
	40-50	6.729 a	5.429	27
	50 year and over	5.719 a	5.321	42
	Overall mean	7.050	5.500	100

TheDifferent capital letters in columns indicate to the significant at 1% level.

The similar small letters in columns indicate to no significant among age groups

The *P27* gene belongs to the recently identified family of proteins called cyclin-dependent kinase inhibitors .These proteins play an important role as negative regulators of cell cycle-dependent kinase activity during progression of the cell cycle. The *P27* can inhibit cell proliferation ,they may have a role as tumor suppressor genes (spirin *et al.*,1996). The *P27* is primarily involved in cell cycle arrest G1 via its ability to bind with and inactivated cyclin E-cdk2 complexes(Coats *et al.*,1996). In this study ,it is found that decreased *P27* expression will prevent cell cycle arrest. The down regulation of *P27* ,with antisense molecules, leads to re-entry into the cell cycle, which leads to cell proliferation it was agreed with the results of the study (Rivard *et al.*,1996).These results are accordance with those results obtained by Hengst and Reed,(1996); . Dahia *et al.*,(1996) have stated that the the protein level of *P27* is down-regulated ,however ,the mRNA levels is not always

suppressed accordingly the reason of this discordance has been ascribed to the (proteolysis system of *P27*),the protein level of *P27* has recently been reported to mainly be regulated through the degradation by ubiquitin-dependent proteolysis. A reduction in the *P27* expressing level is associated with high aggressiveness and poor prognosis in various malignant tumors, including breast cancer(Porter *et al.*,1997; Tsuchiya *et al.*,1999). The decrease of *P27* gene expression maybe prevent apoptosis or arrest cells in G1 phase ,thereby reducing the cytotoxic effect of chemotherapeutic drugs that act on proliferating cells (Brown *et al.*,2004). The decreased expression of *p27* is due to increased proteasome-mediated protein degradation, correlates with poor prognosis of patients and this decrease lead contributes to tumor development by increasing in CDK activity and cell proliferation(Loda et al .,1997;Viglietto et al .,2002).

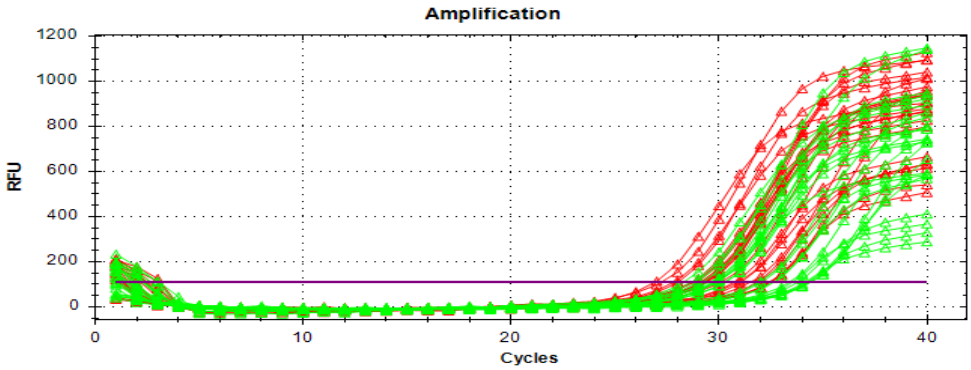

Figure (2):- The Real time amplification plots of p27 gene in patients in green plots and healthy control samples at red plots.

It has also been suggested that a loss of *P27* may desensitize tumor cells to antimitogenic signals ,thus preventing apoptosis in their evolution(Park *et al.*,1999). The principal mechanism of *P27* proteolysis involves its recognition by *Skp2* and subsequent degradation via the ubiquitin pathway. Multiple studies support the independent prognostic significance of *P27*(Catzavelo *et al.*,1997; Chappuis etal .,2000; Tan *et al.*,1997; Han *et al.*,1999; Alkarain and Slingerland ,2003).Loss of p27 activity occurs through accelerated proteolysis, sequestration, and cytoplasmic mislocalization(Alkarain and Slingerland ,2003).(Figure 2)The Real time amplification plots of p27 gene.

No significant effect of age groups and the interaction between treatment and age groups (Table 1). There are decline in gene expression of P27 with age progress, it may due to The effectiveness of p27 gene expression decreases with advancing age. This may be due to the weakening of the body's immunity when advancing with age, thus preventing cell cycle arrest(Rivard *et al.*,1996). The decline in gene expression of P27 with age progress it may due to the weight increase with age. Similar results have been stated by McCampbell *et al.*,(2016).

B-The Recevier Operating Chraracteristic Curve of the *P27* gene expression

Based on the analysis of the results of ROC curve for p27 gene expression (figure 3).it is clear that the area under the curve is 0.844 change fold ,which is good indicator for the detection of breast cancer patients ,as it reached 82% and 23% for sensetivity and specificity,respectively (Table 5).The value of cut-off for p27 gene expression is less than 5.703 change fold,this value means that any women less than 5.703 change fold is patient with breast cancer (Table 5).

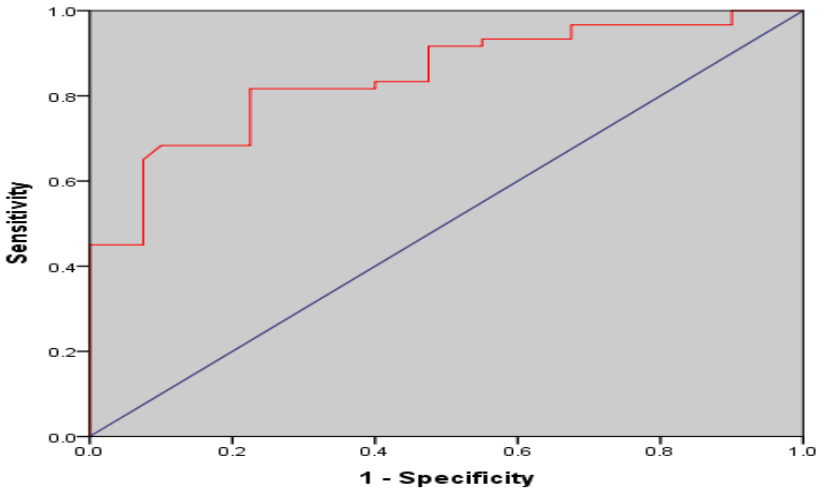

Figure (3):- The recevier operating chraracteristic curve of the P27 gene expression

Table (5) The parameters of the Receiver operating characteretic curve of the P27 gene expression.

parameters	Patients group	Control group
Number	60	40
AUC	0.844	
Standard error	0.038	
95% CI*	0.769-0.919	
Sensivity	81.7%	
Specificity	22.5%	
Cut-off point	⩽ 6.52	
Criterion	True positive 49 False Negative 11 Total positive 42 cases	True negative 31 False negative 9 Total positive 8 cases

D-SKP2 gene expression

Table (6) illustrates the the study groups show a high significant ($p<0.01$) , the patients group gene expression was 13.943 in comparison to control; group which was 3.261.

Table(6) :-The means ± SD of gene expression for SKP2

Study groups	Age groups	Mean	±SD	Number
Patient group	30-40	9.573	6.761	9
	40-50	18.169	27.983	15
	50 year and over	13.275	18.998	36
	Total	13.943 A	20.343	60
Control group	30-40	2.326	2.511	22
	40-50	5.754	4.539	12
	50 year and over	3.261	1.802	6
	Total	3.494 B	3.459	40
Total	30-40	4.4298 a	5.271	31
	40-50	12.651 a	21.676	27
	50 year and over	11.845 a	17.918	42
	Overall mean	9.764	16.668	100

SKP2 targets *P27* for ubiquitination and proteolysis. It is hypothesized that increased levels of *SKP2* will lead to accelerated proteolysis of *P27* and activation of cyclin E– CDK2(Carrano *et al.*,1999). The expression of *SKP2* is low in the G0/G1 phase, but increases in the S phase. Over-expressed Skp2 in the G0/G1 phase could lead to an outof-control G1/S phase, disruption of cell proliferation, and differentiation.(Mamillapalli *et al.*,2001; Haas ,2005).

Gstaiger *et al.*,(2001); Radke *et al.*,(2005) have been found that S phase kinase-associated protein 2 (*SKP2*) was overexpressed in a number of cancer types, including breast cancer, where it aids in the

development of the malignancy. It has been reported that *SKP2* mediates the ubiquitin degradation of several cyclin-dependent kinase (CDK) inhibitors, such as *p21*cip1, *p27*kip1, and *p57*kip2).(Mamillapalli *et al.*,2001; Kamura *et al.*,2003; Bencivenga *et al.*,2014).High levels of *SKP2* protein are associated with ER negativity, low *P27*expression, high proliferation rate, and poor survival.(Signoretti *et al.*,2002). It has been reported that there is a statistically significant association between *SKP2* expression levels and breast tumor grades. Moreover, high expression levels of *SKP2* are associated with poor survival (Signoretti *et al.*, 2002). The increased *SKP2* gene expression maybe by suppressed *P53*-mediated apoptosis,the overexpression of *SKP2* enhanced cell proliferation in normal breast cell line while deplation of *SKP2* reduces cellular growth in breast cancer cell line(kitagawa *et al.*,2008). *SKP2* have been reported to alter the activity of *P53*(Kitagawa *et al.*,2008; Chander *et al.*,2010). Hermeking et al.,(1997); Lee et al.,(2009) ; Zhang et al.,(2015) stated that simultaneous phosphorylation of p53 at serine 15 and 20 increases expression of PIG3 and induces apoptosis. Also, Zhang etal.,(2016) showed that high Skp2 expression positively correlated with poor prognosis of breast cancer,Skp2 could promote breast cancer cell proliferation, inhibit cell apoptosis, change the cell cycle distribution and induce the increased S phase cells and therefore induce cell proliferation in breast cancer cells, suppress PIG3 expression via independent pathways in the breast cancer cells. Skp2 suppressed p53 and inhibited PIG3-induced apoptosis. (Figure 4)The Real time amplification plots of *SKP2* gene.

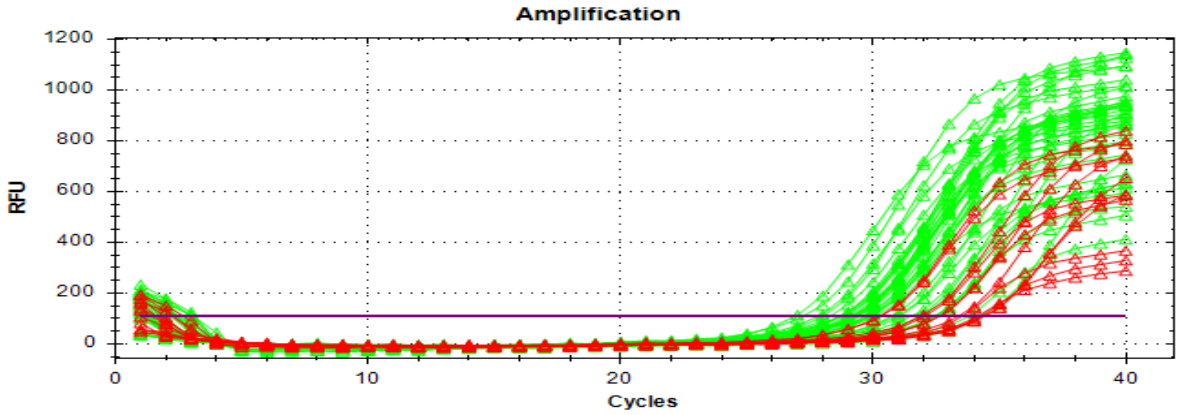

Figure (4):-The Real time amplification plots of SKP2 gene in patients in green plots and healthy control samples at red plots.

The age groups of 40-50 and 50 year and over have achieved the highest gene expression compaired to the gene expression of age group of 30-40 year ,these differences among the different age groups do not reached to significance level (3). There are increase in gene expression of SKP2 with age progress, it may due to High levels of *SKP2* protein are associated with ER negativity, low *P27*expression, high proliferation rate, and poor survival.(Signoretti *et al.*,2002).

E- The Recevier Operating chraracteristic curve of he *SKP2* gene expression

According to the analysis at ROC results for SKP2 gene expression(Figure5) ,the area under curve was 0.754 change fold, this value could be not consider as an efficient area for detection of patients with cancer ,the percentage of sensitivity and specificity was 63% and 37% respectively .The cut-off point was more than 4.486 change fold which mean that any women with value more than 4.486 change fold is patient **(Table 7)**.

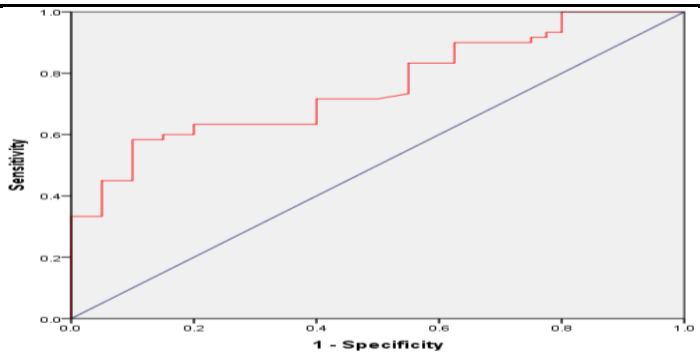

Figure (5):- The recevier operating chraracteristic curve of the SKP2gene expression

Table (7) The parameters of the Receiver operating characteretic curve of the SKP2 gene expression.

parameters	Patients group	Control group
Number AUC Standard error 95% CI Sensivity Specificity Cut-off point Criterion	60 40 0.754 0.047 0.661-0.847 63 % 20% >4.486	
	True positive 38 False Negative 22 Total positive 46 cases	true negative 32 False Negative 8 Total positive 54 cases

References

1. **Alkarain, A., & Slingerland, J. (2003).** Deregulation of p27 by oncogenic signaling and its prognostic significance in **breast** cancer. Breast Cancer Research, 6(1), 1-9.
2. **Bast Jr, R. C., Ravdin, P., Hayes, D. F., Bates, S., Fritsche Jr, H., Jessup, J. M., ... & Somerfield, M. R. (2001).** 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. Journal of clinical oncology, 19(6), 1865-1878.
3. **Bencivenga, D., Tramontano, A., Borgia, A., Negri, A., Caldarelli, I., Oliva, A., ... & Borriello, A. (2014).** P27Kip1 serine 10 phosphorylation determines its metabolism and interaction with cyclin-dependent kinases. Cell cycle, 13(23), 3768-3782.
4. **Benvidi, A., Dehghani Firouzabadi, A., Dehghan Tezerjani, M., Moshtaghiun, S. M., Mazloum-Ardakani, M., & Ansarin, A. (2015).** A highly sensitive and selective electrochemical DNA biosensor to diagnose breast cancer. Journal of Electroanalytical Chemistry, 750, 57–64.
5. **Brown, I., Shalli, K., McDonald, S. L., Moir, S. E., Hutcheon, A. W., Heys, S. D., & Schofield, A. C. (2004).** Reduced expression of p27 is a novel mechanism of docetaxel resistance in breast cancer cells. Breast cancer research, 6(5), 1-7.
6. **Cai, Z., Moten, A., Peng, D., Hsu, C. C., Pan, B. S., Manne, R., ... & Lin, H. K. (2020, December).** The Skp2 pathway: a critical target for cancer therapy. In *Seminars in cancer biology* (Vol. 67, pp. 16-33). Academic Press.
7. **Carrano, A. C., Eytan, E., Hershko, A., & Pagano, M. (1999).** SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. Nature cell biology, 1(4), 193-199
8. **Catzavelos, C., Bhattacharya, N., Ung, Y. C., Wilson, J. A., Roncari, L., Sandhu, C., ... &**

Slingerland, J. M. (1997). Decreased levels of the cell-cycle inhibitor p27 Kip1 protein: prognostic implications in primary breast cancer. Nature medicine, 3, 227-230.

9. **Catzavelos, C., Bhattacharya, N., Ung, Y. C., Wilson, J. A., Roncari, L., Sandhu, C., ... & Slingerland, J. M. (1997).** Decreased levels of the cell-cycle inhibitor p27 Kip1 protein: prognostic implications in primary breast cancer. Nature medicine, 3, 227-230.
10. **Cavallaro, S., Paratore, S., de Snoo, F., Salomone, E., Villari, L., Buscarino, C., Ferraù, F., Banna, G., Furci, M., Strazzanti, A., Cunsolo, R., Pezzino, S., Gangi, S.and Basile, F. (2012).** Genomic analysis: toward a new approach in breast cancer management. Crit. Rev. Oncol. Hematol., 81: 207-223
11. **Chander, H., Halpern, M., Resnick-Silverman, L., Manfredi, J. J., & Germain, D. (2010)**. Skp2B attenuates p53 function by inhibiting prohibitin. EMBO reports, 11(3), 220-225.
12. **Chappuis, P. O., Kapusta, L., Bégin, L. R., Wong, N., Brunet, J. S., Narod, S. A., ... & Foulkes, W. D. (2000).** Germline BRCA1/2 mutations and p27Kip1 protein levels independently predict outcome after breast cancer. Journal of clinical oncology, 18(24), 4045-4052.
13. **Coats, S., Flanagan, W. M., Nourse, J., & Roberts, J. M. (1996).** Requirement of p27Kip1 for restriction point control of the fibroblast cell cycle. Science, 272(5263), 877-880.
14. **Dahia, P. L., Aguiar, R. C., Honegger, J., Fahlbush, R., Jordan, S., Lowe, D. G., ... & Grossman, A. B. (1996).** Mutation and expression analysis of the p27/kip1 gene in corticotrophin-secreting tumours. Oncogene, 16(1), 69-76.
15. **Gstaiger, M., Jordan, R., Lim, M., Catzavelos, C., Mestan, J., Slingerland, J., & Krek, W. (2001).** Skp2 is oncogenic and overexpressed in human cancers. Proceedings of the National Academy of Sciences, 98(9), 5043-5048.
16. **Haas JS. (2005).** Evaluation and outcomes of women with a breast lump and a normal mammogram result. J Gen Intern Med Aug;20(8):692-6.
17. **Habeeb,B.M.(2022)**. Association of ATM and TGFβ1 Genes with Breast Cancer under Radiotherapy in a Sample of Iraqi Women
18. **Han, S., Park, K., Kim, H. Y., Lee, M. S., Kim, H. J., & Kim, Y. D. (1999).** Reduced expression of p27Kip1 protein is associated with poor clinical outcome of breast cancer patients treated with systemic chemotherapy and is linked to cell proliferation and differentiation. Breast cancer research and treatment, 55, 159-165.
19. **Hengst, L., & Reed, S. I. (1996).** Translational control of p27Kip1 accumulation during the cell cycle. Science, 271(5257), 1861-1864.
20. **Heravi Karimovi, M., Pourdehqan, M., Jadid Milani, M., Foroutan, S. K., & Aieen, F. (2006).** Study of the effects of group counseling on quality of sexual life of patients with breast cancer under chemotherapy at Imam Khomeini Hospital. Journal of mazandaran university of medical sciences, 16(54), 43-51.
21. **Hermeking, H., Lengauer, C., Polyak, K., He, T. C., Zhang, L., Thiagalingam, S., ... & Vogelstein, B. (1997)**. 14-3-3σ is a p53-regulated inhibitor of G2/M progression. Molecular cell, 1(1), 3-11.
22. **Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., & Thun, M. J. (2009)**. Cancer statistics, 2009. CA: a cancer journal for clinicians, 59(4), 225-249.
23. **Joyce, C., Rayi, A., & Kasi, A. (2018).** Tumor-Suppressor Genes.
24. **Kamura, T., Hara, T., Kotoshiba, S., Yada, M., Ishida, N., Imaki, H., ... & Nakayama, K. I. (2003).** Degradation of p57 Kip2 mediated by SCFSkp2-dependent ubiquitylation. Proceedings of the National

Academy of Sciences, 100(18), 10231-10236.

25. **Kitagawa, M., Lee, S. H., & McCormick, F. (2008).** Skp2 suppresses p53-dependent apoptosis by inhibiting p300. Molecular cell, 29(2), 217-231.
26. **Lee, J. et al. (2009)** .The function of p27 KIP1 during tumor development. Exp. Mol. Med., 41, 765–771
27. **Lin, H., Ruan, G. Y., Sun, X. Q., Chen, X. Y., Zheng, X., & Sun, P. M. (2019).** Effects of RNAi-induced Skp2 inhibition on cell cycle, apoptosis and proliferation of endometrial carcinoma cells. Experimental and Therapeutic Medicine, 17(5), 3441-3450.
28. **Loda, M., Cukor, B., Tam, S. W., Lavin, P., Fiorentinc, M., Draetta, G. F., ... & Pagano, M. (1997).** Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. Nature medicine, 3(2), 231-234.
29. **Makdissi, F. B. A., Santos, S. S., Bitencourt, A., & Campos, F. A. B. (2022).** An introduction to male breast cancer for urologists: epidemiology, diagnosis, principles of treatment, and special situations. International braz j urol, 48, 760-770.
30. **Mamillapalli, R., Gavrilova, N., Mihaylova, V. T., Tsvetkov, L. M., Wu, H., Zhang, H., & Sun, H. (2001).** PTEN regulates the ubiquitin-dependent degradation of the CDK inhibitor p27KIP1 through the ubiquitin E3 ligase SCFSKP2. Current Biology, 11(4), 263-267.
31. **McCampbell, A. S., Mittelstadt, M. L., Dere, R., Kim, S., Zhou, L., Djordjevic, B., ... & Walker, C. L. (2016).** Loss of p27 associated with risk for endometrial carcinoma arising in the setting of obesity. Current molecular medicine, 16(3), 252-265.
32. **Park, M. S., Rosai, J., Nguyen, H. T., Capodieci, P., Cordon-Cardo, C., & Koff, A. (1999).** p27 and Rb are on overlapping pathways suppressing tumorigenesis in mice. Proceedings of the National Academy of Sciences, 96(11), 6382-6387.
33. **Porter, P. L., Malone, K. E., Heagerty, P. J., Alexander, G. M., Gatti, L. A., Firpo, E. J., ... & Roberts, J. M. (1997).** Expression of cell-cycle regulators p27 Kip1 and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. Nature medicine, 3, 222-225.
34. **Radke, S., Pirkmaier, A., & Germain, D. (2005).** Differential expression of the F-box proteins Skp2 and Skp2B in breast cancer. Oncogene, 24(21), 3448-3458.
35. **Rivard, N., L'Allemain, G., Bartek, J., & Pouysségur, J. (1996).** Abrogation of p27Kip1 by cDNA antisense suppresses quiescence (G0 state) in fibroblasts. Journal of Biological Chemistry, 271(31), 18337-18341.
36. **Russo, A. A., Jeffrey, P. D., Patten, A. K., Massagué, J., & Pavletich, N. P. (1996).** Crystal structure of the p27Kip1 cyclin-dependent-kinase inibitor bound to the cyclin A–Cdk2 complex. Nature, 382(6589), 325-331.
37. **Safaee, A., Zeighami, B., Tabatabaee, H. R., & Moghimi Dehkordi, B. (2008).** Quality of life and related factors in breast cancer patients under chemotherapy. Iranian Journal of Epidemiology, 3(3), 61-66.
38. **Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021.** CA Cancer J Clin. 2021;71:7-33. Erratum in: CA Cancer J Clin. 2021;71:359.
39. **Signoretti, S., Di Marcotullio, L., Richardson, A., Ramaswamy, S., Isaac, B., Rue, M., ... & Pagano, M. (2002).** Oncogenic role of the ubiquitin ligase subunit Skp2 in human breast cancer. The Journal of clinical investigation, 110(5), 633-641.
40. **Singletary, S. E., Allred, C., Ashley, P., Bassett, L. W., Berry, D., Bland, K. I., ... & Greene, F. L. (2003).** Staging system for breast cancer: revisions for the 6th edition of the AJCC Cancer Staging Manual. *Surgical Clinics*, *83*(4), 803-819.
41. **Soerjomataram, I., & Bray, F. (2021).**

Planning for tomorrow: Global cancer incidence and the role of prevention 2020–2070. Nature reviews Clinical oncology, 18(10), 663-672.

42. **Spirin, K. S., Simpson, J. F., Takeuchi, S., Kawamata, N., Miller, C. W., & Koeffler, H. P. (1996).** p27/Kip1 mutation found in breast cancer. Cancer Research, 56(10), 2400-2404.
43. **Spss,(2013).**statistical pachage of social science.version22,aplication Guide:copy Right by spss Inc.USA.
44. **Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020**: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021.
45. **Tan, P., Cady, B., Wanner, M., Worland, P., Cukor, B., Magi-Galluzzi, C., ... & Loda, M. (1997)**. The cell cycle inhibitor p27 is an independent prognostic marker in small (T1a, b) invasive breast carcinomas. Cancer research, 57(7), 1259-1263.
46. **Traub, F., Mengel, M., Lück, H. J., Kreipe, H. H., & von Wasielewski, R. (2006).** Prognostic impact of Skp2 and p27 in human breast cancer. Breast cancer research and treatment, 99, 185-191.
47. **Tsuchiya, A., Zhang, G. J., & Kanno, M. (1999).** Prognostic impact of cyclin-dependent kinase inhibitor p27kip1 in node-positive breast cancer. Journal of surgical oncology, 70(4), 230-
48. **Viglietto, G., Motti, M. L., & Fresco, A. (2002).** Understanding p27kip1 deregulation in cancer: downregulation or mislocalizaiton?. Cell cycle, 1(6), 394-400.Visvader, J. E., & Stingl, J. (2014). Mammary stem cells and the differentiation hierarchy: current status and perspectives. Genes & development, 28(11), 1143-1158.
49. **Wang, Z., Fukushima, H., Inuzuka, H., Wan, L., Liu, P., Gao, D., ... & Wei, W. (2012).** Skp2 is a promising therapeutic target in breast cancer. Frontiers in oncology, 1, 57.
50. **World Health Organization ,2020.**Estimated of the new cases in 2020,worldwide New Global Cancer Data.(WHO,2020).
51. **Zhang, W., Cao, L., Sun, Z., Xu, J., Tang, L., Chen, W., ... & Guan, X. (2016)**. Skp2 is over-expressed in breast cancer and promotes breast cancer cell proliferation. Cell cycle, 15(10), 1344-1351.
52. **Zhang, W., Luo, J., Chen, F., Yang, F., Song, W., Zhu, A., & Guan, X. (2015).** BRCA1 regulates PIG3-mediated apoptosis in a p53-dependent manner. Oncotarget, 6(10), 7608.Zhang, W., Cao, L., Sun, Z., Xu, J., Tang, L., Chen, W., ... & Guan, X. (2016). Skp2 is over-expressed in breast cancer and promotes breast cancer cell proliferation. Cell cycle, 15(10), 1344-1351.
53. **Zweignd,M.H.,campbell.G.(1993).**Receiver-operating characteristic (ROC) plots : a fundamental evaluation tooin clinical medicine .clinical chemistry.,39:561-577.