Vol 5 Issue 10 Nov 2015

ISSN No : 2230-7850

International Multidisciplinary Research Journal

Indian Streams Research Journal

Executive Editor
Ashok Yakkaldevi

Editor-in-Chief
H.N.Jagtap

Welcome to ISRJ

RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

Indian Streams Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial board.Readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

International Advisory Board

Editorial Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.org

Indian Streams Research Journal
ISSN 2230-7850

Impact Factor : 3.1560(UIF)
Volume - 5 | Issue - 10 | Nov - 2015

EXPRESSION OF HER2 AND TOPOII ALPHA IN BREAST CANCER

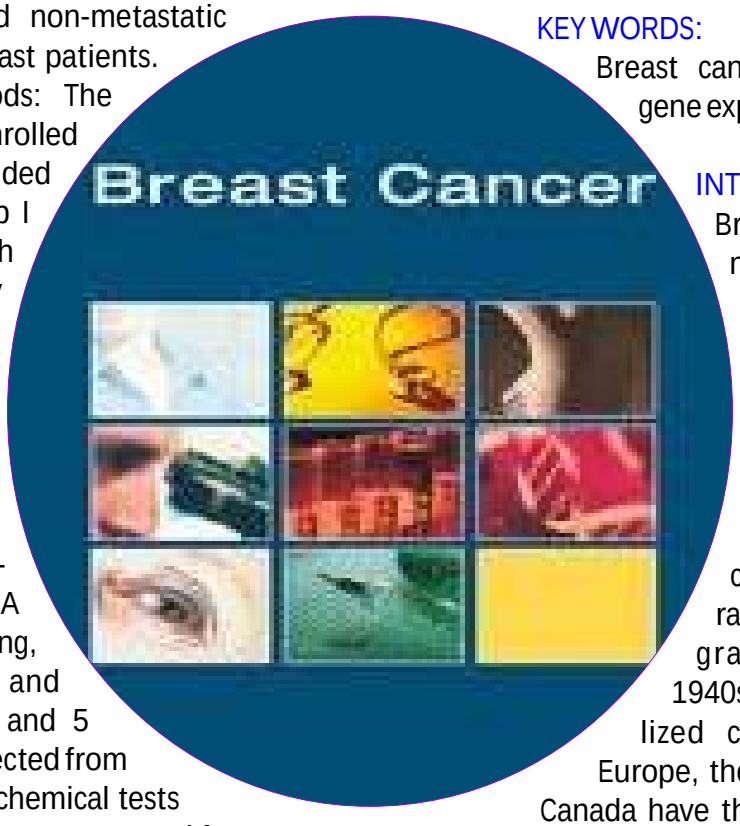
[1]Diab,M. , [2]Shaker, O. , [2]Nassar, Y. , [1]Abd El-Aziz, G. and [3]El-Marzoki,M.
[1]Medical Biochemistry, Faculty of Medicine, Beni Suef University.
[2]Medical Biochemistry, Faculty of Medicine, Cairo University.
[3]General surgery, Faculty of medicine, Cairo University, Egypt.

ABSTRACT:

Background: In breast cancer (BC) overexpression of some oncogenes as Human epidermal growth factor receptor and Topoisomerase IIA has been shown to play an important role in the development and progression of certain aggressive types. In recent years these proteins have become an important biomarkers. Objective: The aim of this study was to detect the expression of Her2 and TopoIIA genes in both metastatic and non-metastatic groups of cancer breast patients. Subject and Methods: The present study was enrolled on 230 subjects divided into 3groups: Group I (control) which included 50 healthy subjects. Group II which included 40 patients of metastatic breast cancer cases Group III which contains 140 patients with non-metastatic breast. A detailed history taking, thorough physical and clinical examination and 5 ml of blood were collected from each subject and biochemical tests were done. Total RNA was extracted from whole blood using RNA extraction kit and then reverse transcription was done followed by real time PCR. Results: The study showed significant correlation between control and non-metastatic cancer groups as regards' Her2 and TopoIIA expression (p<0.001*) & (p<0.001*) respectively and between control and metastatic groups as regards' Her2 and TopoIIA expression (p<0.001*) & (p<0.001*) respectively and significant correlation between each other.Conclusion: Her2 and TopoIIA gene expressions are important diagnostic markers for non-metastatic and for anticipation of metastatic breast cancer.

KEY WORDS:

Breast cancer, Her2, TopoIIA, gene expression.



INTRODUCTION:

Breast cancer is the most common cancer among women and is second only to lung cancer as a cause of cancer death among women in the developed world.Breast cancer incidence rates have increased gradually since the 1940s in many industrialized countries. Western Europe, the United States, and Canada have the highest incidence of breast cancer, with the lowest rates found in Asia (Parkin *et al*., 2001).In Egypt, breast cancer is estimated to be the most common cancer

among females in 2008. It is also the leading cause of cancer-related mortality accounting for 29.1% of their total death .The incidence to mortality ratio is poor (1.9:1).These estimates are confirmed in many regional Egyptian cancer registries as well as in hospital-based frequencies (The National Cancer Registry Program of Egypt, 2012).There are several clinically recognized types of breast cancer, with ductal carcinoma the most prevalent, followed by lobular carcinoma, inflammatory breast cancer, medullary carcinoma of the breast, and other less common forms (Turashvili *et al*., 2007).

Reduction in mortality from BC depends on interventions aimed at early detection and treatment, including breast self-examination, clinical breast examination, and mammography. Lack of early detection programs is the primary reason for the escalation of the mortality rate from BC in developing countries (Sadler *et al*., 2001).Her2 is a member of the epidermal growth factor receptor (EGFR/ERBB) family. Amplification or overexpression of this oncogene has been shown to play an important role in the development and progression of certain aggressive types of breast cancer. In recent years the protein has become an important biomarker and target of therapy for approximately 30% of breast cancer patients (Mitri *et al*., 2012).The Her2 gene is located at chromosome 17q21 and thegene is amplified in 20-30% of breast carcinomas. Close tothe Her2 locus is the gene encoding for topoisomerase IIa(TopoIIA) at 17q21-q22. The enzyme TopoIIA is an importantcomponent in DNA replication with the capability ofmaking a double-stranded break in the DNA helix, allowingunwinding of DNA coils before sealing the strandstogether again for continued replication (Coon *et al*., 2002).

DNA topoisomerases are enzymes that regulate the over winding or under winding of DNA and are required for the survival of all organisms. They play an important role in regulating cellular processes such as replication, transcription, and chromosomal segregation by altering DNA topology (Schoeffler *et al*., 2008).DNA topoisomerase IIA (TopoIIA) is a well-known anticancer target. Agents that target TopoIIA are among the most effective anticancer drugs currently available for the treatment of human cancers. TopoIIA gene is located adjacent to the Her2 oncogene at the chromosome 17. TopoIIA is amplified in almost 90% of Her2 amplified primary breast tumors (Azarova et al., 2007). Therefore, the aim of our study was to detect the expression of Her2 and TopoIIA genes in both metastatic and non-metastatic groups of breast cancer patients compared with healthy subjects

SUBJECTS AND METHODS

The present study included one hundred eighty (180) Egyptian women with age ranged from (20-65 years). They were recruited from National Cancer Institute in the period from January 2011 to December 2013. Patients was known as breast carcinoma according to history taking, clinical examination and confirmed by mammography and surgical biopsies. Fifty (50) healthy controls recruited during routine checkup, who were proven to be healthy with no family history of breast cancer.A written informed consent was obtained from patients to participate in the study in accordance with the ethical guidelines of the Declaration of Helsinki.

The studied subjects were divided into three groups: Group I: (n=50) healthy females as a control and Group II (n=40) patients with metastatic breast cancer cases (30 invasive ductal and 10 invasive lobular carcinoma), all 40 patients with bone metastasis, 36 with axillary and 4 supraclavicular lymphnode involvement, and according to grading system38 cases are grade 2 and 2 grade3.Group III: (n=140), this group include 140 patients with non-metastatic breast cancer, they are classified into (12 invasive lobular and 128 invasive ductal carcinoma) and according to TNM grading system into (88 cases with T2 and 52 cases with T3, also 108 cases were grade 2 and 32 cases were grade 3).

Analytic procedure:

Venous blood samples were collected from patients and controls in sterile EDTA and centrifuge tubes. Part in the centrifuge tube was incubated at 37°c for 15 minutes then centrifuged at 3,000 rpm for 10 minutes at room temperature, serum was separated and used for biochemical tests and the rest of blood was taken for RNA extraction and detection of gene expression of Her2 and TopoIIA.
Quantitation of Her-2 and topoisomerase II α by real time PCR

1. Total RNA extraction:

Total RNA was extracted from whole blood using RNA extraction kit provided by Qiagene extraction kit. The RNA purity and concentration were quantified by NanoDrop ND-1000 (Nanodrop, USA).

2. Reverse transcription:

Reverse transcription for the extracted RNA was done using SuperScript™ II reverse transcriptase (Invitrogen Life Technologies Inc., Carlsbad, CA). The reaction was carried out for 60 min at 42°C and the reaction mixture was subsequently inactivated for 15 min at 70°C. The cDNA was stored at -70°C till used for quantitativePCR.

3. Primers probe kits:

Primers and TaqMan probes for Her-2 and the GAPDH control reference gene were designed andsynthesized according to Taqman Gene Expression Assay (assays Hs00170433_m1 and 4326317E, respectively) (Applied Biosystems, Foster City, CA, USA). The sequence of the primers of topoisomerase II A was Forward primer.

5'-TTGAAGACGCTTCGTTATGGG-3', Reverse primer 5'-CCATCACAACTGGCCCTCTC-3' and Probe 5'-ACAGATCAGGACCAAGATGGTTCCCACAT-3'

4. PCR for Her-2 mRNA and topoisomerase II a :

Polymerase chain reaction (PCR) was performed in a final volume of 20µL. In which1.25ul primer probesmixture, 1.25 ul GAPDH, 5ul cDNA and 2.5 ul H2O.The reaction was carried out using real time PCR for 45 cycles of: 95°C for 15 sec and 60?C for 30 sec.The relative quantification (RQ) was given by the ratio between the mean value of the target gene and the mean value of the reference gene (GAPDH) in each sample. The relative amount of PCR product generated from each primer set was determined on the basis of the cycle threshold (Ct) value. The RQ was calculated by 2- ??CT. HER-2 and topoisomerase II a relative expressions level were compared with the healthy controls.

Statistical analysis:

Data were statistically described in terms of minimum, maximum, mean, standard deviation, median, frequencies (number of cases) and relative frequencies (percentages) when appropriate. Comparison of quantitative variables was done using Mann-Whitney test when comparing two subgroups and Krauscal-Wallis when comparing more than 2 subgroups. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Receiver operator characteristic (ROC) curves were derived and area-under-the curve (AUC) analysis performed to get the best cutoff value of Her-2 and of Topoisomerase II alpha for detecting cancer cases and metastasis. A probability value (P value) less than 0.05 was considered

statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 21.

RESULTS

Two hundred thirty (230) Egyptian women with age ranged from (20-65 years). Showing thatthere was a significant difference between metastatic cases in breast cancer as regards grade, type and lymphnode involvement (p<0.001*, p<0.05 and p<0.05) respectively. Also, showing significant relationship between non metastatic cases in breast cancer as regards type (p<0.05) and estrogen/progesterone receptors (p<0.05), while no significance as regards grade, and family history(Table 1).

Table 1: Grade, type, family history, ER/PR and Lymphnode in both metastatic and non-metastatic breast cancer groups.

Parameters		Metastasis			Non-metastasis		
		Count	%	P value	Count	%	P value
Grade	2	38	95%	<0.001*	108	78.4%	0.215
	3	2	5%		32	21.6%	
Type	Invasive duct	30	75%	<0.05*	128	91.8%	<0.05*
	invasive lobular	10	25%		12	8.2%	
Family history	No	-	-	-	68	45.5%	0.572
	Yes	-	-		72	54.5%	
ER/PR	Negative	-	-	-	118	84.1%	<0.05*
	Positive	-	-		22	15.9%	
Lymphnode	Axillary	36	90%	<0.05*	-	-	-
	Supraclavicular	4	10%		-	-	

* indicates a statistical significant difference.

As regards grade, type and lymphnode involvement (p<0.001*, p<0.05 and p<0.05) respectively (Table 2) showed significance in metastatic cases in breast cancer.

Table 2: Her2 and TopoIIA expression in non-metastatic, metastatic breast cancer and control groups.

Parameters		Control group	Non-metastatic group	Metastasis group	P value
Her-2	Mean	0.70	8.45	26.10	<0.001*
	Standard Deviation	0.29	11.97	20.92	
TopoIIA	Mean	0.64	5.80	12.26	<0.001*
	Standard Deviation	0.28	6.58	9.26	

* indicates a statistical significant difference

As regards ER/PR (estrogen/progesterone receptors) expression and menstrual history there was a statistically significant difference (median=12.8, minimum=0.8, maximum=25.3),

(median=0.8,minimum=0.1, maximum=22.7) respectively, (pvalue =0.002&0.004, respectively), and TopoIIA expression(Table 3).Also,Showed that, there was a statistically significant difference between Her2 expression as regards ER/PR (estrogen/progesterone receptors) (median =18.6, minimum =1.05, maximum= 63.4) and (p<0.0001*) and menstrual history (median=14.3,median=0.7,maximum=75.5) and (p=0.007*)(Table 3).

Table 3: TopoIIA and Her2 expression in relation to ER/PR and menstrual history in all breast cancer groups.

Parameters		TopoIIA			P value	Median	Her2		
		Median	Minimum	Maximum			Minimum	Maximum	P value
ER/PR	-ve	2.3	0.30	25.70	0.002*	3.4	0.50	51.45	<0.0001*
	+v	12.8	0.80	25.30		18.6	1.05	63.40	
Menstrual history	post	6.4	0.50	42.20	0.004*	14.3	0.70	75.50	0.007*
	pre	0.8	0.10	22.70		0.85	0.10	66.70	

* indicates a statistical significant difference

As shown in (Table 4) there was non-significant difference between Her2 expression as regards grade, lymph nodes, type, and family history. While there was significant relationship as regards expression of TopoIIA as regard family history, but there was no significant relationship as regards grade, LN and type in metastatic group of breast.

Table 4:Her2 and TopoIIA expression in relation to grade, lymph nodes, typeand family history in metastatic breast group.

Parameters		Her-2				TopoIIA			
		Median	Minimum	Maximum	P value	Median	Minimum	Maximum	P value
Grade	2	7.77	0.56	75.50	0.767	4.30	0.40	42.20	0.628
	3	4.15	0.50	60.40		3.10	0.30	22.70	
Lymph nodes	Axillary	18.40	6.30	75.50	0.801	12.60	2.40	42.20	0.208
	supraclavicular	38.50	10.30	66.70		5.15	4.80	5.50	
Type	Invasive duct	5.77	0.50	75.50	0.492	4.00	0.30	42.20	0.780
	invasive lobular	9.83	0.80	18.60		2.80	0.40	14.20	
Family history	No	4.98	0.56	63.40	0.157	4.30	0.40	25.30	0.042*
	Yes	3.40	0.50	51.45		2.20	0.30	20.70	

* indicates a statistical significant difference

As regard clinical size (p <0.04*), Showedsignificance correlation as regards TopoIIA(Fig. 1), While no significant correlation as regards Her2 and clinical size in metastatic group (Table5).

Table 5: Correlation between Her2 and topoisomerase IIA as regard clinical size in metastatic cancer breast group.

Parameters	Clinical size		
	N	P value	r
Her2	20	0.99	0.379
TopoIIA	20	<0.04*	0.463

* indicates a statistical significant difference

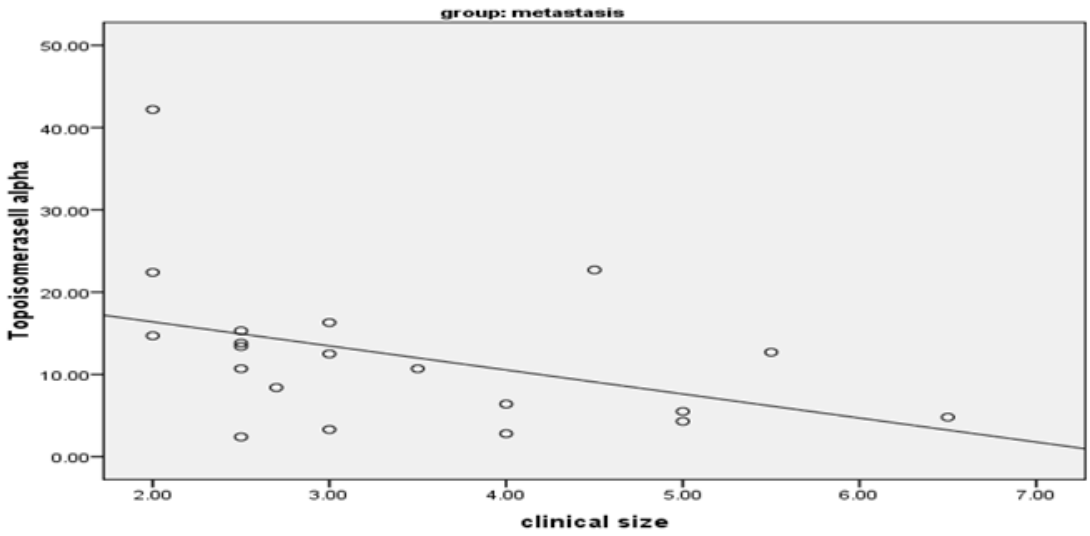


Fig. 1: Showed significant correlation between TopoIIA as regard clinical size in metastatic group.

Also, (Table 6) and (Fig. 2) Showed that, there was a statistically significant difference between TopoIIA expression as regard Her-2 expression (p <0.001). And also was a statistically significant difference between Her-2 expression as regard TopoIIA expression (p <0.001).

Table6: Correlation between Her-2 with TopoIIA expression in breast cancer.

Parameter	TopoIIA		
	N	P value	r
Her2	90	<0.001*	0.879

* indicates a statistical significant difference

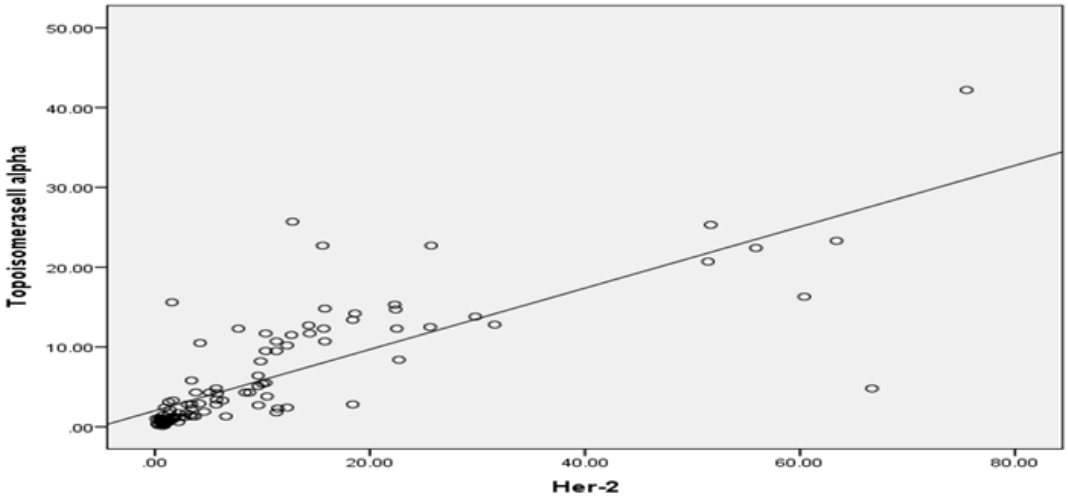


Fig. 2: Showing significant correlation between Her2 and TopoIIA expression in breast cancer patients.

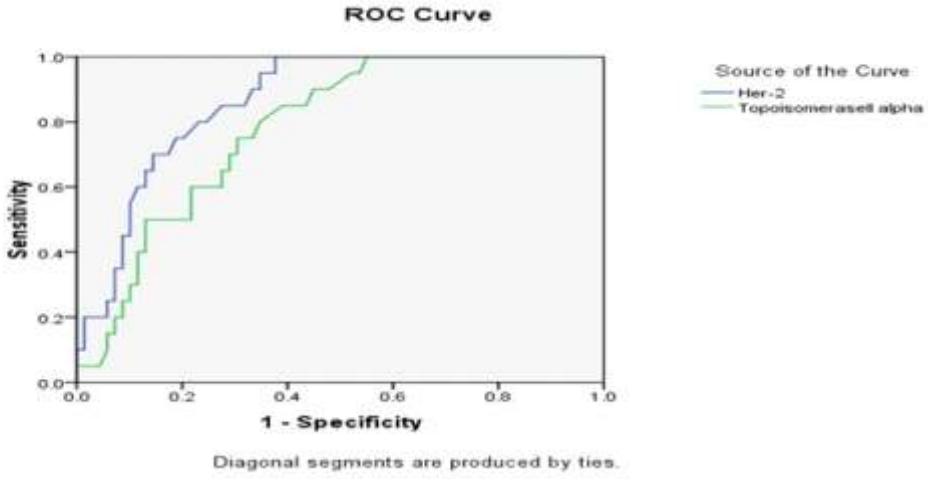


Fig. 3: ROC curve of her2 & TopoIIA in metastatic breast cancer.

Table 7: Her2 &TopoIIA expression in metastatic breast cancer.

Test Result Variable(s)	Area under curve	P value	95% Confidence Interval
Her-2	0.865	<0.001*	0.790-0.940
TopoIIA	0.782	<0.001*	0.685-0.879

Parameters	Sensitivity	Specificity
Her2	100%	62.30%
TopoIIA	90%	55.1%

The best cutoff value of her-2 = 6.05 with sensitivity 100% and specificity 62.3 %
The best cutoff value of topoisomerase IIA = 3.2 with sensitivity 90% and specificity 55.1%.

DISCUSSION

Her2 is a member of the epidermal growth factor receptor (EGFR/ERBB) family. Amplification or overexpression of this oncogene has been shown to play an important role in the development and progression of certain aggressive types of breast cancer. In recent years the protein has become an important biomarker and target of therapy for approximately 30% of breast cancer patients (Mitri *et al*., 2012).DNA topoisomerase IIA (TopoIIA) is a well-known anticancer target. Agents that target TopoIIA are among the most effective anticancer drugs currently available for the treatment of human cancers (Azarova *et al*., 2007). The enzyme TopoIIA is an importantcomponent in DNA replication with the capability ofmaking a double-stranded break in the DNA helix, allowingunwinding of DNA coils before sealing the strandstogether again for continued replication (Coon *et al*., 2002).

The present study showed significant difference in non-metastatic cases in breast cancer as regard type ($p<0.05$) and estrogen/progesterone receptors ($p<0.05$) meanwhile, no significant difference as regards grade and family history, also,there is significant difference between metastatic cases in breast cancer as regard grade ($p<0.001$), type ($p<0.05$) and lymph node ($p<0.05$).

This agreed with Putti*et al*. (2005), who stated that, as estrogens play such a significant role in mammary gland and tumor development, breast tumors are frequently characterized as ERα positive or negative. This is explained byGupta and Kuperwasser (2006), who stated that, normal growth and development of mammary epithelial tissue were under the regulation of estrogens. Estrogens are steroid hormones that interact with intracellular estrogen receptors ERa and ERß to activate gene transcription via estrogen-response elements (EREs) located near the promoter regions of estrogen-responsive genes. While Zhou *et al.* (2002), explained that, Similar to estrogen, progesterone action includes regulation of gene expression differentially modulated by the two receptor isoforms such that PR-B activates transcription and PR-A is transcriptionally inactive and acts as a transdominant repressor of PR-B. Further, PR-A is involved in repression of ER, androgen receptor (AR), and PR-B dependent gene expression.

This disagreed with Foulkes and Narod (1995), who stated that, about 5 to 10% of breast cancers in the general population have a hereditary basis. In affected families, however, risk is particularly high if a first-degree relative has premenopausal bilateral breast cancer or two first-degree relatives have any form of breast cancer. The major known breast cancer genetic mutations, BRCA1 and BRCA2, are involved in DNA repair and transcriptional regulation.In our study there was no significant correlation between Her2 and TopoIIA and clinicopathological parameters (grade, LN, TNM grading system), however there was significant correlation between TopoIIA and tumor size ($p<0.04$). These results coincided with Todorovi *et al*. (2009), who detected that neither Her2 nor TopoIIA amplification has shown correlation with available clinicopathological parameters such as age, menopausal status, steroid receptor status (estrogen and progesteron receptors), tumor size, nodal status, distant metastases, histologic type and stage at time of diagnosis.These results agreed also with Lindemann *et al*. (2007), who stated that there was no correlation between Her2 overexpression of tumor tissue and other clinicopathological findings. While Zhu *et al*. (2008), has reported that, high TopoIIA expression was associated with poor histological grade, high proliferative activity. Several studies analyzed these correlations with different subsets and achieved the same results.

While this result disagreed with Depowski *et al*.(2000), who reported that increased Topo IIA expression is correlated with clinicopathological features such as (decreased patient survival (p 5 .001),

advanced tumor stage (p= 0 .034), lymph node metastasis (p= 0 .018), Tumor stage (p < .0001), node-positive status (p < .0001) and tumor grade (p 0 .025).Also our result disagreed with Gianni et al. (2011), who stated that the prognosis of patients with TN tumors remains poor as no new therapeutic agents targeting TN tumors are available, and also patients with luminal tumors exhibited a relatively good prognosis. No metastasis, other than brain metastasis, was observed in patients with the Her2 disease subtype.This is explained by Park et al. (2006), also explained that Her2 amplification is an early event in human breast tumorigenesis. Her2 amplification is seen in nearly half of all in situ ductal carcinomas without any evidence of invasive disease. Her2 status is maintained during progression to invasive disease, nodal metastasis and distant metastasis.The results of this study revealed that there was significant correlation between Her2 and TopoIIA as regards HR status(ER/PR) (< 0.0001* and 0.002*) respectively.

This finding agrees with Zaczek and Sparano (2012), who found strong associationbetween TopoIIA copy number change and HR and Her2status. Tokoiniwa *et al*. (2012) evaluated ER-positive, Her2-negative breast cancer or Topo IIA expression. They found 46% cases were positive for Topo IIA overexpression. Rody *et al.* (2009) reported that 48% of cases were positive in ER-positive subset.In contrast, Depowski *et al*.(2000), found no correlation with topo IIA expression and tumor size, tumor grade, ER status, PR status, or disease recurrence.

In our study there was significant correlation between control and both non metastatic cancer group and metastatic group as regards' Her2 and TopoIIA expression (p<0.001*). About TopoIIA, this agreed with Depowski *et al*. (2000), who denoted that increased topo II expression is associated with an aggressive form of breast cancer featuring Her-2 expression and predicts disease-related death, lymph node metastasis, and advanced tumor stage. And also this agreed with Woessner *et al*. (1991), who said that TopoIIA was essential for cell growth and was typically expressed at high levels in rapidly growing cancer cells, whereas TopoIIß is expressed in quiescent cells in virtually all tissues throughout the whole cell cycle and was dispensable for cell survival.This was explained by the fact that TopoIIA is a ubiquitous ribozyme that alters the instantaneous cleavage of double-stranded DNA and the chromosomal topological structure, facilitating subsequent double-strand break (DSB) religation. These enzymes are involved in many aspects of DNA metabolism, including DNA replication, transcription, repair, and chromosome condensation/ segregation Toyoda et al. (2008).

As regards Her2,this was agreed with Owens et al. (2004), who stated that Human epidermal growth factor receptor 2 (Her2) is a gene amplified or overexpressed in 20%–25% of all breast cancers Her2 positivity is associated with an aggressive disease course, a poor prognosis, and relative sensitivity to anthracyclines.Lindemann *et al*. (2007), also performed Immunohistochemistry for Her2 and revealed overexpression in 20 cases (51.3%), whereas 19 cases were scored negative. No Her2 expression was observed in normal ducts. Immunofluorescent staining for Her2 showed positive immunofluorescence intensities in both normal and tumor tissue.

This is explained by Holbro*et al*. (2003), who stated that increased Her2 homodimers disrupt cell polarity. Increased dimers drive proliferative, survival, invasive and metabolic functions. Increased Her2 expression results in an increase in the rare DHer2 isoform withmore potent signaling characteristics. Several transcription factors are induced in Her2-overexpressing cells resulting in a plethora of gene expression changes.

This study revealed also, that there was significant correlation between expression of both Her2 and TopoIIA with each other in the all groups of breast cancer cells (P<0.001?).This finding agreed with Depowski et al. (2000), who reported that there was increased TopoIIA expression correlated with Her2 expression (p < 0.0001) .This finding also agreed with Rody et al. (2009), Zaczek et al. (2012) and

Sparano *et al.* (2012).The most important finding in the previous studies was the strong associationbetween TopoIIA copy number change and HR and Her2status.

Also obtained results agreed with Hicks and Tubbs (2005), who reported that inHer2-positive breast cancer, amplification of TopoIIA variesfrom 25% to 42%. WhileJacot *et al*. (2013) assessed that, 29.1% of the Her2-amplifiedcases were co-amplified with TopoIIA. The proportion of TopoIIApositive tumors in this study was lower than in other studies. However, the frequency of Her2 amplification is comparablewith others, and this weighs against methodological problems. TopoIIA aberrationsare strongly associated with HR and Her2 status.In contrast to Jarvinen *et al*. (2000) and Karen *et al*. (2004), who had shown that amplifications of HER2 and TopoIIA are independent events.

The results of this study were coincided by Jacot et al. (2013), who denoted that TopoIIA is one of the genes close to HER2 and its protein product, topoisomerase II A, is the molecular target of anthracycline treatment. TopoIIA amplification status has been thought to be linked to response to treatment. However, data are conflicting and, as yet, unresolved ,and also explained with Karen *et al*. (2004), who showed that the status of the TopoIIA gene has gained interest as a predictive marker for treatment with anthracyclines and for the close relationship to HER2, both topographically on the chromosome 17 and pathologically with frequent co expression.

Further, the obtained results showed significant value of Her2 and TopoIIA in anticipation of metastatic group of breast cancer ($p<0.001$*& $p<0.001$*) respectively with 95% confident interval (0.790-0.940&0.685-0.879) respectively, with the best cutoff value of Her2 = 6.05 with sensitivity 100% and specificity 62.3 % .The best cutoff value of TopoIIA = 3.2 with sensitivity 90% and specificity 55.1%.Also, showed significant value of Her2 and TopoIIA as predictors of non-metastatic group of breast cancer ($p<0.001$*& $p<0.001$*) respectively with 95% confident interval (0.926-0.992&0.894-0.979) respectively, with The best cutoff value of Her2 = 1.225 with sensitivity 88.8% and specificity 100 % The best cutoff value of TopoIIA = 1.15 with sensitivity 84% and specificity 100%.This agreed with Depowski *et al*.(2000) who stated that the correlation of TopoIIA expression with other known prognostic parameters has been inconsistent.Indeed, TopoIIA was accepted as strictly proliferation marker in breast cancer, mostly because of its role and high expression in proliferating cells.

Also the results agreed with Coon *et al*. (2002) who detected the correlation between Her2 amplifications and TopoIIA genecopy number changes inpreclinical and early clinical studies, it has been speculatedthat TOPIIA is in fact the predictive marker for chemotherapy with anthracyclines.Karen *et al*. (2004) showed that the status of the TopoIIA gene has gained interest as a predictive marker for treatment with anthracyclines and for the close relationship to Her2, both topographically on the chromosome 17 and pathologically with frequent co expression.

In contrast, Jacot *et al*. (2013) stated that as a prognostic marker, TopoIIA is probably of limited value. TopoIIA aberrations are strongly associated with HR and Her2 status, and the importance of these markers in prognostication is still unchallenged.

This is explained by the fact that over expression of the Her2 protein, either through gene amplification or through transcriptional deregulation is seen in approximately 25–30% of breast and ovarian cancers and confers worse biological behavior. Initial conflicting reports regarding the prognostic relevance of Her2 were resolved with improved methodologies and the overwhelming data now confirms this initial landmark genetic-biologic finding. Breast cancers can have up to 25–50 copies of the Her2 gene and up to 40- to 100-fold increase in Her2 protein expression resulting in up to 2 million receptors expressed at the tumor-cell surface Lohrisch and Piccart (2001).

CONCLUSION:

Her2 and TopoIIA genes are over expressed in both metastatic and non-metastatic breast cancer groups and also have significant relationship as regards expression of each other, so Her2 and TopoIIA genes expression are important diagnostic markers for non-metastatic breast cancer cases and a good marker for anticipation of metastatic breast cancer cases.

LIST OF ABBREVIATIONS:

BC:Breast cancer
Her2:Human epithelial growth factor receptor
TopoII A:Topoisomerase 2 alpha
EGRF:Epidermal growth factor receptor
PCR:Polymerase chain reaction
ER:Estrogen receptor
HR:Hormonal receptor
PR:Progesterone receptors
AR:Androgen receptor
ERE: Estrogen releasing elements
BRCA1:Breast cancer gene one
DSB:Double strand break
DHer2:Dimer human epidermal growth factor receptor
RQ:Relative quantitation
ELISA: Enzyme- Linked-ImmunosorbentAssay.
EDETA: Ethelene diamine tetra acetic acid.

ACKNOWLEDGMENTS:

The authors would like to thank all members of the staff at Medical Biochemistry and Molecular Biology Department, Cairo University for their help and support and the Department of General Surgery at Qasr El Aini hospital for contributing to the data collection and health examinations.

REFERENCES

1.Azarova, A.M., Lyu, Y.L., Lin, C.P., Tsai, Y.C., Lau, J.Y., Wang, J.C. and Liu, L.F. 2007. Roles of DNA topoisomerase II isozymes in chemotherapy and secondary malignancies. Proc. Natl. Acad. Sci. USA., 104: 11014-11019.
2.Coon, J.S., Marcus, E. and Gupta-Burt, S. 2002. Amplification and overexpression of topoisomerase IIalpha predict response to anthracycline-based therapy in locally advanced breast cancer. Clin. Cancer Res., 8: 1061-1067.
3.Depowski, S.L., Rosenthal, T.M., Brien, S., Stylis, R.L., Johnson and J.S. Ross 2000. Topoisomerase 2 alpha expression in breast cancer: correlation with clinical variables, Mod Pathol., 13: 542-547.
4.Foulkes, W., and Narod, S. 1995. Hereditary breast and ovarian cancer: Epidemiology, genetics, screening and predictive testing. Clin.Invest. Med., 18: 473-483.
5.Gianni, L., Dafni, U. and Gelber, R.D. (2011): Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with Her2.positive early breast cancer: a 4year follow up of a randomised controlled trial. Lancet Oncol 12: 236-244.
6.Gupta, P. B., and Kuperwasser, C. 2006. Contributions of estrogen to ER-negative breast tumor growth. J. Steroid Biochem. Mol. Biol., 102: 71-78.

7.Hicks, D.G. and Tubbs, R.R. 2005. Assessment of the Her2 status in breast cancer by fluorescence in situ hybridization: a technical review with interpretive guidelines. Hum Pathol., 36: 250-261.
8.Holbro, T., Beerli, R.R., Maurer, F., Koziczak, M., Barbas, C.F. and Hynes, .N.E. 2003. The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. Proc. Natl. Acad. Sci. USA, 100: 8933-8938.
9.Jacot,W., Fiche, M. and Zaman, K. 2013. The Her2 amplicon in breast cancer: Topoisomerase IIA and beyond. Biochim. Biophys. Acta., 1836: 146–57.
10.Jarvinen, T.A., Tanner, M and Rantanen, V. 2000. Amplification and deletion of topoisomerase IIalpha associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. Am. J. Pathol., 156: 839-847.
11.Karen, E., Olsen., Helle- Knudsen., Birgitte, B., Rasmussen, Eva- Balslev., Ann-Knoop., Bent-Ejlertsen, Kirsten ,V. and Jens- Overgaard. 2004. for the Danish Breast Cancer Co-operative Group Acta Oncologica, 43(1): 35-42.
12.Lindemann,L., Resau., Na-hrig., Leeser., Annecke., Welk,. Scha., Lengyel and Harbeck, N. 2007. Histopathology, 51: 54-62.
13.Lohrisch, C. and Piccart, M. (2001): An overview of Her2. Semin Oncol 28: 3–11.
14.Mitri,Z., Constantine, T. and O'Regan, R. 2012. "The Her2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy". Chemother. Res. Pract. doi:10.1155/2012/743193.
15.Owens, M.A., Horten, B.C. and Da Silva, M.M. 2004. Her2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. Clinical Breast Cancer, 5: 63-69. doi: 10.3816.
16.Parkin, D., Bray, F. and Devesa, S. 2001. Cancer burden in the year 2000. The global picture. Eur. J. Cancer., 37: S4-S66.
17.Putti, T. C., El-Rehim, D. M., Rakha, E. A., Paish, C. E., Lee, A. H., Pinder, S. E. and Ellis, I. O. 2005. Estrogen receptor-negative breastcarcinomas: A review of morphology and immunophenotypical analysis. Mol. Pathol., 18: 26-35.
18.Rody, A, Karn T, Ruckhaberle E, 2009. Gene expression of topoisomerase II alpha(TOP2A) by microarray analysis is highly prognostic in estrogen receptor (ER) positivebreast cancer. Breast Cancer Res Treat., 113: 457-66.
19.Sadler, G.R., Dhanjal, S.K., Shah, N.B., Ko, C.M. and Anghel, M. 2001. Asian India women: Knowledge, attitudes and behaviours toward breast cancer early detection. Public Health Nurs., 18: 357-363.
20.Schoeffler, A.J. and Berger, J.M. 2008. DNA topoisomerases: Harnessing and constraining energy to govern chromosome topology. Q Rev. Biophys., 41: 41-101.
21.Sparano, J.A., Goldstein, L.J., Davidson, N.E. 2012. TOP2A RNA expression andrecurrence in estrogen receptor-positive breast cancer. Breast Cancer Res. Treat., 134: 751–7.
22.The National Cancer Registry Program of Egypt (NCRPE) 2012. Reports and Statistics. Journal of the Egyptian National Cancer Institute, 27: 1-5.
23.Todorovi, C., Rakovi, Z. and Nikoli-Vukosavljevi. 2009. Metastatic breast cancer survival according to HER2 and Topo2a gene status Disease Markers, 26: 171–180.
24.Tokiniwa, Horiguchi., Takata., Kikuchi., Rokutanda., Katsunori, T., Tetsunari, O. and Izumi, Takeyoshi 2012. Breast Cancer, 19: 309-314.
25.Turashvili, G., Bouchal, J., Baumforth, K., Wei, W., Dziechciarkova, M., Ehrmann, J., Klein, J., Fridman, E., Skarda, J., Srovnal, J.,Hajduch, M., Murray, P., and Kolar, Z. 2007. Novel markers for differentiation of

lobular and ductal invasive breast carcinomas bylaser microdissection and microarray analysis. BioMed Central (BMC) Cancer, 7: 55.

26.Woessner, R.D., Mattern, M.R., Mirabelli, C.K., Johnson, R.K. and Drake, F.H. 1991. Proliferation- and cell cycle-dependent differences in expression of the 170 kilodalton and 180 kilodalton forms of topoisomerase II in NIH-3T3 cells. Cell Growth Differ., 2: 209-214.

27.Zaczek, A, Markiewicz A, Supernat A. 2012. Prognostic value of TOP2A gene amplificationand chromosome 17 polysomy in early breast cancer. Pathol. Oncol. Res., 18: 885–94.

28.Zhou, H., Luo, M. P., Schönthal, A. H., Pike, M. C., Stallcup, M. R., Blumenthal, M., Zheng, W. and Dubeau, L. 2002. Effect of reproductive hormones on ovarian epithelial tumors: I. Effect on cell cycle activity. Cancer Biol. Ther., 1: 300-306.

29.Zhu, L., Li, Y.F., Chen, W.G., He, J.R., Peng, C.H. and Zhu, Z.G. 2008. HER2 and topoisomerase IIa: possible predictors of response to neoadjuvant chemotherapy for breast cancer patients. Chin. Med. J., 121: 1965–68.

Publish Research Article
International Level Multidisciplinary Research Journal
For All Subjects

Dear Sir/Mam,

We invite unpublished Research Paper,Summary of Research Project,Theses,Books and Book Review for publication,you will be pleased to know that our journals are

Associated and Indexed,India

- International Scientific Journal Consortium
- OPEN J-GATE

Associated and Indexed,USA

- Google Scholar
- EBSCO
- DOAJ
- Index Copernicus
- Publication Index
- Academic Journal Database
- Contemporary Research Index
- Academic Paper Databse
- Digital Journals Database
- Current Index to Scholarly Journals
- Elite Scientific Journal Archive
- Directory Of Academic Resources
- Scholar Journal Index
- Recent Science Index
- Scientific Resources Database
- Directory Of Research Journal Indexing

Indian Streams Research Journal
258/34 Raviwar Peth Solapur-413005,Maharashtra
Contact-9595359435
E-Mail-ayisrj@yahoo.in/ayisrj2011@gmail.com
Website : www.isrj.org