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Original Article

Evaluation of Immunohistochemical Expression of Topoisomerase II Alpha Protein in Patients with Breast Cancer and Its Correlation with Different Prognostic Factors

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Abstract

Background: There are indications to support the knowledge about the prognosis of breast cancer. One of these markers is regarded as a prognostic factor and related to the proliferation rate. Topoisomerase IIα (TOP2A), encoded by the TOP2A gene, is a molecular target for anthracycline therapy. **Aims:** The aim of this article is to establish any association of the status of topoisomerase IIa immunohistochemistry with different prognostic clinicopathological and molecular parameters in breast cancer. **Settings and Design:** A cross-sectional retrospective study was carried out in the Department of Pathology and Forensic Medicine, Faculty of Medicine, Babylon University, during the period from January 2020 through December 2020. **Materials and Methods:** Fifty cases with invasive breast carcinoma have been obtained from surgical modified radical mastectomy specimens from the Laboratory of Histopathology in Al-Kafeel Specialized Hospital, Karbala for the last 3 years (2017–2019). Different clinicopathological variables were estimated. The expression of TOP2A was stained by using the PathnSitu PolyExcel Detection System of Immunostaining. **Statistical Analysis Used:** Immunostaining with TOP2A expression used a cut-off value of 10%. The results were considered statistically significant if the *P*-value was ≤ 0.05. **Results:** Fifty cases of TOP2A overexpression were classified as follows: 28 cases (56%) had 3+ expression, 4 cases (8%) with 2+ expression, and 4 cases (8%) with 1+ expression, whereas 14 cases had no expression of TOP2A (28%). TOP2A overexpression was shown to be associated with a higher grade ($P = 0.023$) and molecular subtypes ($P = 0.057$), but not with other clinicopathological parameters (age of patients, type of histology, number of lymph nodes involved, tumor size). **Conclusion:** According to these results, TOP2A plays a major role in the aggressive nature of tumors. This may support the suggestion to be used as a prognosis marker.

Keywords: Breast cancer, breast cancer prognosis, topoisomerase IIa (TOPA2)

Introduction

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death among women.

Worldwide, in 2020, female breast cancer has now surpassed lung cancer as the leading cause of global cancer incidence in both sexes combined, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases. It is the fifth leading cause of cancer mortality worldwide. So among women, breast cancer accounts for one in four cancer cases and one in six cancer deaths.[1]

In Iraq, in 2020, breast cancer is the first one to rank among the malignancies in all populations; it accounted for 22.2% of total cancer cases and 37.9% of all new cancer cases among females of all ages. Breast cancer ranks the first among the total cancer deaths, and it is established as 15.3%.[2]

The highest incidence of mortality from cancer in females was breast cancer (4.79/100,000 female populations).[3]

Breast cancers show considerable variation in the sense of their histological features and molecular alterations, and

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such factors are known to influence the patient outcome and clinical behavior.[4]

The changes in molecules that classify breast carcinogenesis have been established. Therefore, four subtypes of breast cancer were distinguished by group tumors: luminal A, luminal B, enriched human epidermal growth factor receptor 2 (HER2), and triple-negative breast cancer (basal-like).[5] This classification has changed from tumor burden to biology-centered approaches to clinical management of breast cancer.[6]

Recognition of best prognostic and predictive indicators is the main challenge in clinical oncology practice. For that reason, selection of better techniques for evaluating each indicator will influence the tumor therapy and prognosis.[7]

DNA topoisomerase IIa (TOP2A) is a nucleic enzyme that affects the topological structure of DNA by interacting with the double-helix DNA, thus playing an important role in DNA replication, transcription, recombination, condensation, and segregation.[8]

TOP2A expression in tumor cells is reported to be a direct molecular target for anthracycline-containing therapy; cells with a lower degree of an enzyme are less sensitive to the drug and cells containing a high amount are more responsive.[9]

There is growing evidence that TOP2A protein expression is a strong prognostic factor, and TOP2A gene amplification might be a predictive marker (particularly used for anthracyclines therapy). Therefore, it is considered as a prognosis marker for breast cancer aggressiveness.[10]

Materials and Methods

This study is a cross-sectional retrospective study carried out in the Department of Pathology and Forensic Medicine, Faculty of Medicine, University of Babylon, Iraq, during the period from January 2020 through December 2020.

Specimen selection and data collection

Study group

Fifty formalin-fixed and paraffin-embedded tissue blocks from archived tissue specimens of female patients diagnosed with invasive breast carcinoma have been obtained from surgical modified radical mastectomy specimens from the Laboratory of Histopathology in Al-Kafeel Specialized Hospital, Karbala for the last 3 years (2017–2019).

The primary diagnosis was established by using slides stained with (hematoxylin/eosin). Furthermore, molecular subclassification was done by re-examination of the slides stained with immunohistochemistry (IHC) for ER, PR, and HER2, which were all extracted from the archive.

The cases were evaluated by two pathologists, and also the authors re-examined them for histological diagnosis, pathological parameters including lymph node status, tumor grade, tumor size, histological subtypes, and IHC (ER, PR, and HER-2).

The selection of cases

Sample from patients with axillary dissection diagnosed as carcinoma with invasion to or beyond the basement membrane, regardless of histological classification (ductal or lobular or other types), different ages, grades, stages, molecular subtypes, was selected.

Cases with true cut biopsy, lumpectomy, and radical mastectomy specimens were excluded due to deficient data regarding the parameters of involved lymph nodes and tumor size. Furthermore, cases with total lymph node numbers less than 10 in axillary clearance were excluded.

New 5 μm thickness histological sections were made from each of the paraffin-embedded blocks fixed on positively charged slides to be processed and stained to be subjected to conduct IHC procedures to detect topoisomerase IIA.

Control group

The internal control group involved 10 blocks consisting of known five positive and five negative tissues from patients with non-breast cancer, whereas an external control group included two blocks of tonsils and spleen. All are used for topoisomerase IIA IHC evaluation.

I-Positive control

Parallel positive control sections were processed with each set of immunostaining, which is known to express topoisomerase IIa, and were used with each run.

II-Negative controls

Sections untreated with primary antibody (topoisomerase IIa) were considered as negative controls for each set of slides.

Ethical considerations

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local Ethics Committee according to the document number 88 on the 23rd of November 2020.

Results

Clinicopathological parameters of patients with breast cancer

The distribution of patients by age ranged from 20 to 90 years. The overall (mean ± SD) patients' age was 52.06 ± 12.89 years; most of the patients are between fifth

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and sixth decades (32%) 16 cases for each one, whereas decades of third (4%) 2 cases, fourth (12%) 6 cases, seventh (10%) 5 cases, eighth (8%) 4 cases, and ninth (2%) 1 case.

The distribution of patients by tumor-related factors

Grade

Seventeen (34%) cases were grades I and II (66%) and 33 cases had grade III.

Type of histology

Forty-two (84%) cases had ductal non-specific type carcinoma in the breast, 5 (10%) cases had lobular and 3 (6%) cases had different histological types of breast cancer.

According to (ER, PR, Her2) IHC-based molecular subtypes, 34 (68%) cases had luminal A, 4 (8%) cases had luminal B, 6 (12%) cases of Her2-enriched, and 6 (12%) cases had basal-like (triple-negative).

Tumor size

Seventeen (34%) cases had tumor size equal to or less than 20 mm, 30 (60%) cases of tumor size between 21 and 50 mm, and 3 (6%) cases had tumor size >50 mm.

The number of axillary lymph nodes involved

About 34% of the cases had metastasis of lymph node numbers between 1 and 3 nodes involved, 14% of the cases had metastasis of lymph node numbers between 4 and 9, and 10% had lymph node metastasis numbers 10 or more. The excluded total lymph nodes number in axillary clearance is less than 10. These parameters are shown in Table 1.

Table 1: Clinicopathological parameters of patients with breast cancer

Variable	Frequency (%)
Patient age	
20–30	2 (4%)
31–40	6 (12%)
41–50	16 (32%)
51–60	16 (32%)
61–70	5 (10%)
71–80	4 (6%)
81–90	1 (2%)
Tumor grade	
Grades I and II	17 (34%)
Grade III	33 (66%)
Histopathological types	
Ductal	42 (84%)
Lobular	5 (10%)
Other	3 (6%)
Immunohistochemical-based molecular subtypes	
Luminal A	34 (68%)
Luminal B	4 (8%)
Her2-enriched	6 (12 %)
Basal-like (triple negative)	6 (12 %)
Tumor size	
Equal to or less than 20 mm (T1)	17 (34 %)
21–50 mm (T2)	30 (60 %)
More than 50 mm	3 (6 %)
Lymph node metastasis number	
No lymph nodes metastasis	21 (42%)
pN1 (1–3)	17 (34%)
pN2 (4–9)	7 (14%)
pN3 (10 or more)	5 (10%)

IHC results in breast carcinoma

IHC-positive results for topoisomerase IIa were determined as nuclear staining of malignant cells and accordingly, we categorized the results by using *H*-score, which is a semi-quantitative assessment that involves the nuclear staining intensity and percentage of stained tumor cells.

Accordingly, the *H*-score ranged from 0 to 300 in this study.

The study results were recorded as positive when *H*-score was cut-off by more than 10%.

Topoisomerase IIa in breast carcinoma at that score was 36 out of 50 cases (72%). All positive staining results were non-focal, and in most cases, 64% (32/50) show diffuse moderate-to-strong staining [Table 2 and Figure 1].

The result of the study

There were 28 cases (56%) with staining intensity 3+ expression in topoisomerase II alpha which represents 225–300 from *H*-score as in Figure 2, 4 cases (8%) with 2+ expression which represents 150–225 from *H*-score as in Figure 3, and 4 cases (8%) with 1+ expression which represents 10–150 from *H*-score as in Figure 4, whereas 14 cases had no expression of TOP2 II alpha (28%), which was less than 10% of cut-off value of *H*-score as in Figure 5.

Note that we found strong brown cytoplasmic staining in invasive papillary carcinoma.

The association between age of patients with breast cancer and topoisomerase IIa expression

IHC analysis of topoisomerase IIa expression with ages of patients having breast carcinoma revealed that (+3) 28 cases were reported more common (about 9 cases) in each age between 41 and 50, 8 cases in ages between 51 and 60, whereas (+2) 4 cases were reported to be more common (about 2 cases) in ages 31 and 40 and (+1) 4 cases were reported to be more common (about 2 cases) in ages 51 and 60. Out of the 14 cases, negative expressions were reported more commonly in about 6 cases in each age range 41–50 and 51–60, as shown in Figure 6. No significant association between topoisomerase IIa immunostaining and age was found, as shown in Table 3.

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Table 2: Topoisomerase IIa immunoreactivity in breast carcinoma at variable *H*-score cut-off values

H-score cut-off	No. of breast carcinoma cases	Topoisomerase IIa sensitivity
> 10	36/50	72%
> 75	36/50	72%
> 150	32/50	64%
> 225	28/50	56%

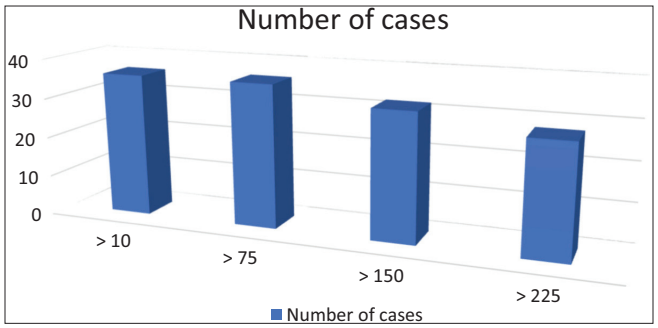

Figure 1: Topoisomerase IIa reactivity in breast carcinoma at variable *H*-score cut-off values

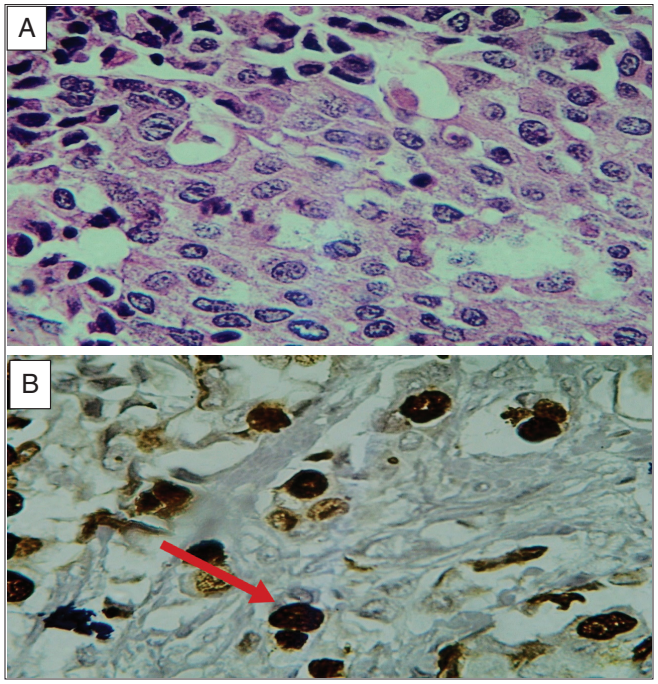

Figure 2: Invasive ductal carcinoma non-specific type (NST), grade 3. (a) H&E-stained slide section (×400). (b) Topoisomerase 2a-stained slide section with (*H*-score >225) strong +3 dark brown nuclear (red arrow) (×400)

The association between grade of breast cancer and topoisomerase IIa expression

IHC analysis of topoisomerase IIa expression with a grade of breast carcinoma revealed that 17 (34%) cases

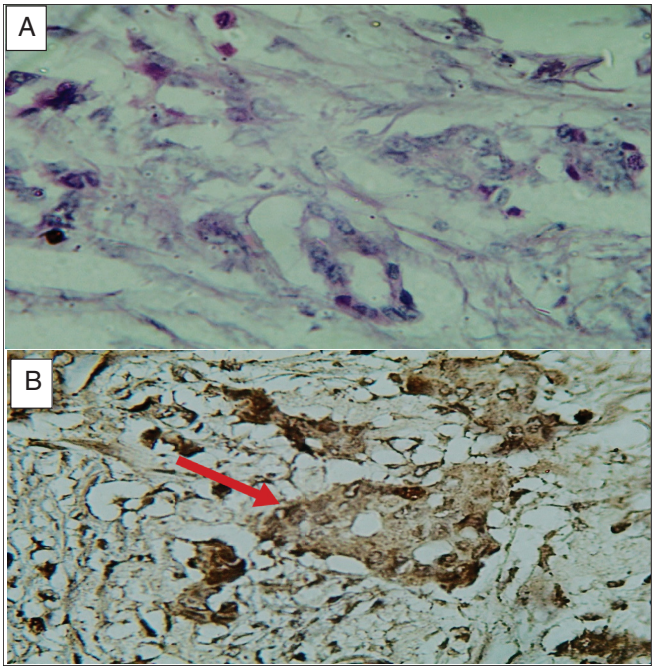

Figure 3: Invasive ductal carcinoma non-specific type (NST), grade 1. (a)H&E-stained slide section (×400). (b) Topoisomerase 2a-stained slide section with (*H*-score between 150 and 225) moderate +2 brown nuclear (red arrow) (×400)

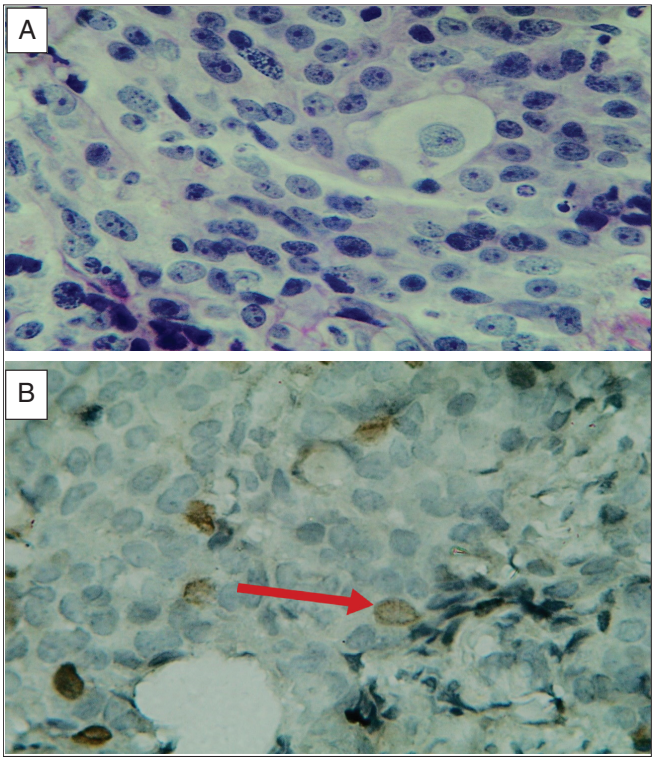

Figure 4: Invasive ductal carcinoma non-specific type (NST), grade 2. (a) H&E-stained slide section (×400). (b) Topoisomerase 2a-stained slide section with (*H*-score between 10 and 150) mild +1 faint brown nuclear (red arrow) (×400)

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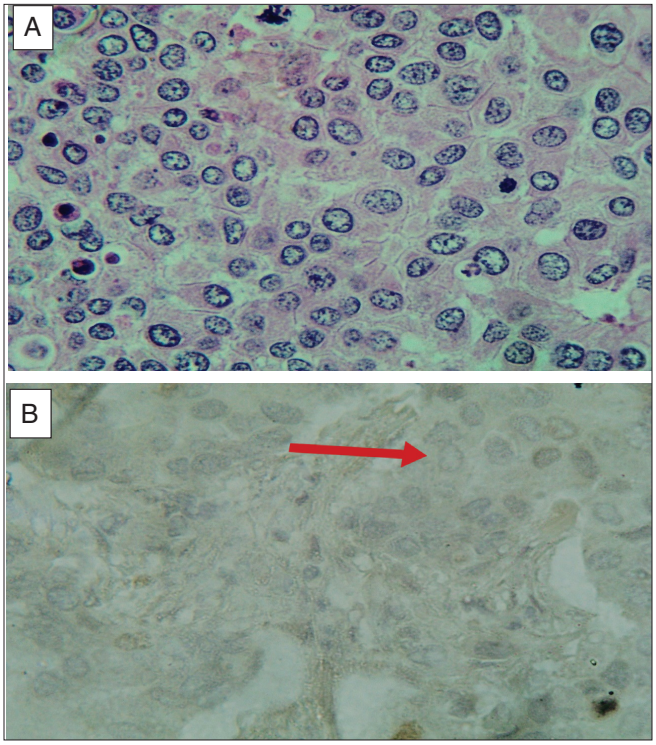

Figure 5: Invasive ductal carcinoma non-specific type (NST), grade 3. (a) H&E-stained slide section (×400). (b) Topoisomerase 2a-stained slide section with (*H*-score <10) (negative) loss of stain expression (red arrow) (×400)

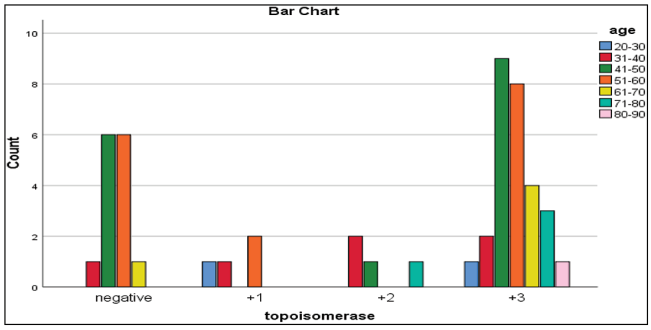

Figure 6: Association between the age of patients with breast cancer and topoisomerase IIa expression

were grades I and (66%) II, and 33 cases had grade III including the following.

- (+3) was reported in 8 cases of grades I and II and 20 cases of grade III tumors.
- (+2) was reported in 2 cases of grades I and II and 2 cases of grade III tumors.
- (+1) was reported in 4 cases of grades I and II and no cases of grade III tumors.
- The negative expression was reported in 3 cases of grades I and II and 11 cases of grade III tumors. There is a significant association between topoisomerase IIa immunostaining and the grade of the tumor, as shown in Table 4.

The association between histology types of breast cancer and topoisomerase IIa expression

IHC analysis of topoisomerase IIa expression with a type of histology revealed the following:

- (84%) 42 cases had a ductal type of breast carcinoma, including (+3) 24 cases, (+2) 3 cases, and (+1) 4 cases;
- (10%) five cases had a lobular type, with topoisomerase IIa including (+3) three cases, whereas (+1) and (+2) no case;
- (6%) three cases had different histological types of breast cancers, (+1) and (+2) had one case;
- 14 cases had no expression of topoisomerase IIa including 11 ductal cases, 2 lobular cases, 1 other case as in Figure 7.

No significant association between topoisomerase IIa immunostaining and types of histology is shown in Table 5.

The association between (ER, PR, Her2) IHC-based molecular subtypes of breast cancer and topoisomerase IIa expression

Topoisomerase IIa expression in (ER, PR, Her2) IHC-based molecular subtypes includes the following:

- (68%) 34 breast cancer cases had luminal A including (+3) 20 cases, (+2) 3 cases, (+1) no case;
- (8%) 4 cases had luminal B including (+3) 2 cases, (+1) 2 cases, and (+2) no case;
- (12%) 6 cases had Her2-enriched including (+3) 4 cases, (+2) no case, and (+1) 1 case;
- (12%) 6 cases had basal-like (triple-negative), (+3) 3 cases, (+2) 2 cases, and (+1) no case;
- no topoisomerase IIa expression in 14 cases, including 11 cases luminal A, no case luminal B, 2 cases Her2-enriched, 1 case triple-negative.

A *significant* association was found between topoisomerase IIa immunostaining and (ER, PR, Her2) IHC-based molecular subtypes of the tumor, as shown in Table 6.

The association between the number of axillary lymph nodes involved in breast cancer and topoisomerase IIa expression

IHC analysis of topoisomerase IIa expression with number of axillary lymph nodes involved is as follows:

- lymph node metastasis numbers between 1 and 3 nodes were involved in 17 (34%) cases with topoisomerase IIa expression being (+3) in 10 cases, (+2) 1 case, and (+1) 2 cases;
- lymph node metastasis numbers between 4 and 9 were involved in 7 (14%) cases with topoisomerase IIa expression being (+3) in 4 cases, (+2) no case, and (+1) 1 case;

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Table 3: Association between topoisomerase IIa expression and the age of patients

		Topoisomerase IIa				Total	*P*-value
		Negative, *H*-score less than 10	+1, *H*-score (10–150)	+2, *H*-score (150–225)	+3, *H*-score (225–300)		
Age	20–30	0	1	0	1	2	0.286 No significant
	31–40	1	1	2	2	6	
	41–50	6	0	1	9	16	
	51–60	6	2	0	8	16	
	61–70	1	0	0	4	5	
	71–80	0	0	1	3	4	
	80–90	0	0	0	1	1	
Total		14	4	4	28	50	

Table 4: Association between topoisomerase IIa expression and the grades of breast cancers

		Topoisomerase IIa				Total	*P*-value
		Negative, *H*-score less than 10	+1, *H*-score (10–150)	+2, *H*-score (150–225)	+3, *H*-score (225–300)		
Histopathology	Ductal	11	4	3	24	42	0.644 No significant
	Lobular	2	0	0	3	5	
	Other	1	0	1	1	3	
Total		14	4	4	28	50	

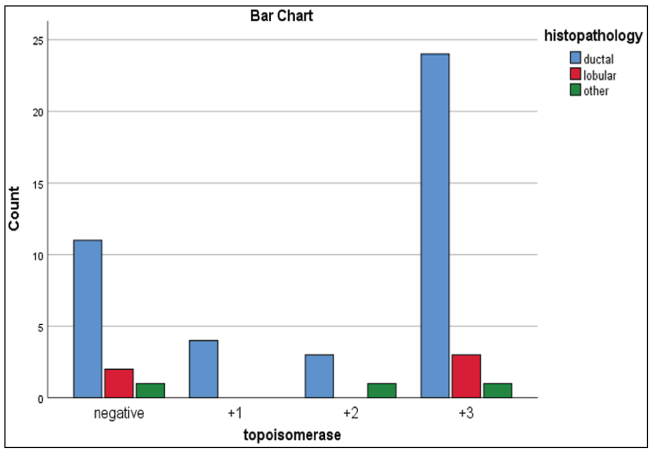

Figure 7: Association between histological type breast cancer and topoisomerase IIa expression

- lymph node metastasis numbers 10 or more were involved in 5 (10%) cases with topoisomerase IIa expression being (+3) in 3 cases and (+1) and (+2) no cases;
- 21 (42%) cases of no involvement of lymph nodes had an expression of topoisomerase IIa, which were about (+3) in 11 cases, (+2) 3 cases, (+1) 1 case;
- no expression of topoisomerase IIa in 4 cases in pN1 (1–3) lymph nodes, 2 cases in pN2 (4–9) lymph nodes, 2 cases in pN3 (10 or more) lymph nodes, and 6 cases with no lymph nodes, as in Figure 8.

No significant correlation was found between topoisomerase IIa immunostaining and the number of lymph nodes involved, as shown in Table 7.

The association between tumor size of breast cancer and topoisomerase IIa expression

IHC analysis of topoisomerase IIa expression with tumor size is as follows:

- (34%) 17 cases had a tumor size equal to or less than 2 cm (T1), with an expression of topoisomerase IIa about (+3) being 9 cases, (+2) 2 cases, and (+1) 1 case;
- (60%) 30 cases had a tumor size between 21 and 50 mm (T2), with an expression of topoisomerase IIa about (+3) being 17 cases, (+2) 2 cases, and (+1) 3 cases;
- (6%) 3 cases had a tumor size >50 mm, with the expression of topoisomerase IIa about (+3) being 2 cases, (+1) and (+2) no cases, as shown in Figure 9.

No significant correlation was found between topoisomerase IIa immunostaining and size of the tumor, as shown in Table 8.

Discussion

Breast cancer is the most prevalent form of cancer in women worldwide, and it is also the leading cause of cancer mortality in women. It has a huge burden on the global health authorities.

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Table 5: Association between Topoisomerase IIa expression and types of histology of breast cancer

		Topoisomerase IIa				Total	*P*-value
		Negative, *H*-score less than 10	+1, *H*-score (10–150)	+2, *H*-score (150–225)	+3, *H*-score (225–300)		
Grade	I and II	3	4	2	8	**17**	0.023 Significant
	III	11	0	2	20	**33**	
Total		14	4	4	28	50	

Table 6: The association between (ER, PR, Her2) immunohistochemical-based molecular subtypes of breast cancer and topoisomerase IIa expression

		Topoisomerase IIa				Total	*P*-value
		Negative, *H*-score less than 10	+1, *H*-score (10–150)	+2, *H*-score (150–225)	+3, *H*-score (225–300)		
(ER, PR, Her2) immunohistochemical-based molecular subtypes	Luminal A	11	0	3	20	34	0.057 Significant
	Luminal B	0	2	0	2	4	
	HER2-enriched	2	1	1	2	6	
	Basal-like (triple-negative)	1	1	0	4	6	
Total		14	4	4	28	50	

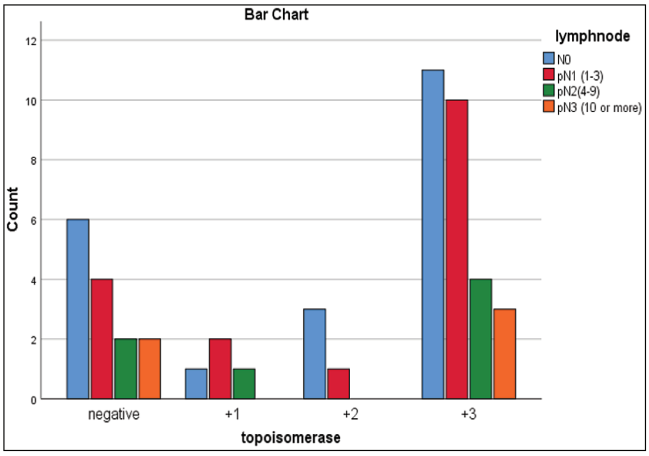

Figure 8: Association between topoisomerase IIa expression and lymph nodes involved with breast cancer

The most commonly used and the simplest way to detect the TOP2A protein expression is IHC, although no standard antibodies, methods of staining, or scoring system was recommended.[11]

This study presents 50 cases of breast cancers graded using the Nottingham Histologic Score System and staged according to TNM (TNM is a system to describe the amount and spread of cancer in a patient's body. T describes the size of the tumor and any spread of cancer into nearby tissue; N describes spread of cancer to nearby lymph nodes; and M describes metastasis [spread of cancer to other parts of the body].)/American Joint Committee on Cancer (AJCC) to test multiple clinical-pathological parameters with TOP2A expression (age, grade, molecular subtypes, histopathological forms, size of tumor, lymph node number involved).

A number of studied cases and different cut-off values used to define overexpression, whether 5%,[12] 10%,[8,13,14] 15%,[7] 20%,[15] 25%,[15] and 30%,[11] have been used in various studies.

TOP2A expression was identified through IHC in up to 50–80% of the cases (detection according to the 10% cut-off).[13-15]

In the current analysis, 10% cut-off value is used to define overexpression. We noted a strong positive association between the staining strengths of nuclear TOP2A in the positive cells, and the TOP2A expression identified about 72% (36 cases with TOP2A overexpression from 50 cases), which was consistent with worldwide studies in the same subject.

There is still debate about the prognostic effect of TOP2A protein expression on breast cancer, and the role of TOP2A in breast cancer prognosis is uncertain.

Some studies have shown that overexpression of TOP2A has been connected to poor outcomes.[16,17] Others have proved a strong effect.[9,11] In contrast, others who failed to show an association of this form have reported a restricted prognostic effect.[18]

This difference among studies could be explained by different antibody clones used, whether monoclonal or polyclonal with different specificities and sensitivities; the absence of the standard procedure and the description of

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Table 7: Association between Topoisomerase IIa expression and number of axillary lymph nodes involved in breast cancer

		Topoisomerase IIa				Total	*P*-value
		Negative, *H*-score less than 10	+1, *H*-score (10–150)	+2, *H*-score (150–225)	+3, *H*-score (225–300)		
Lymph nodes	N0	6	1	3	11	21	0.919 No significant
	pN1 (1–3)	4	2	1	10	17	
	pN2 (4–9)	2	1	0	4	7	
	pN3 (10 or more)	2	0	0	3	5	
Total		14	4	4	28	50	

Table 8: Association between topoisomerase IIa expression and number of lymph nodes involved in breast cancer

		Topoisomerase IIa				Total	*P*-value
		Negative, *H*-score less than 10	+1, *H*-score (10–150)	+2, *H*-score (150–225)	+3, *H*-score (225–300)		
Tumor size	Less than 20 mm (T1)	5	1	2	9	17	0.975 No significant
	21–50 mm (T2)	8	3	2	17	30	
	More than 50 mm (T3)	1	0	0	2	3	
Total		14	4	4	28	50	

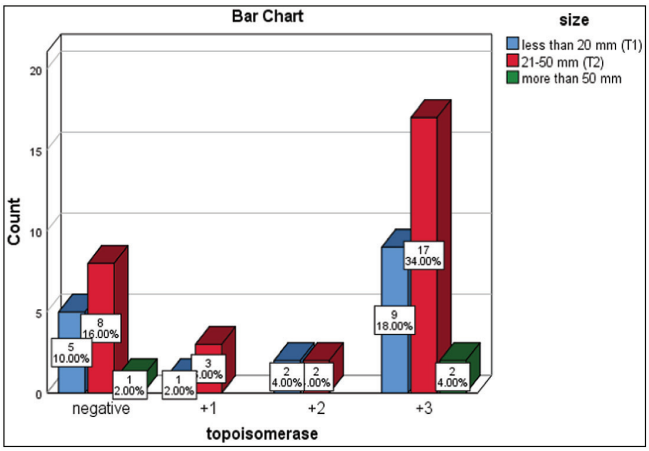

Figure 9: Association between the size of breast cancer and topoisomerase IIa expression

IHC of TOP2A protein, as described earlier, may be one explanation. Another possible cause might be owing to either using automated analysis of quantitative images[7] or using subjective analysis[12,13] for the measurement of protein expression.

The different prognostic effects of protein TOP2A for different subtypes of breast cancers may be another possible reason.

The intensity of staining and the proportion of positive cells in the TOP2A status given have been taken into account in other studies.[19]

To help in such a debate, we evaluated the association of TOP2A expression with some prognostic clinicopathological parameters.

There is significant evidence that breast cancer is a heterogeneous disease rather than a single disease, and there are breast cancer subtypes that appear with different prognoses, i.e., specific clinicopathological characteristics.[15,20,21]

This study evaluated TOP2A expression protein with some of these parameters.

We found that higher grade tumor was strongly linked with the expression of the TOP2A protein, suggesting that tumors with a higher TOP2A expression were more aggressive; this is consistent with some previous studies that stated that the protein TOP2A was significantly linked to a high grade of the tumor[10,11] and inconsistent with other different studies.[9,19]

In our study, TOP2A expression (ER, PR, Her2) IHC-based molecular subtyping was evaluated in breast cancer women who expressed it: (68%) 34 cases had luminal A, (8%) 4 cases had luminal B, (12%) 6 cases had Her2-enriched, (12%) 6 cases had basal-like (triple-negative), which verified that there was a significant expression of protein TOP2A related to (ER, PR, Her2) IHC molecular subtypes. This was consistent with studies that stated that protein, TOP2A, was substantially linked to molecular subtypes[15] and separated ER and PR individually,[9,12,14] HER-2 status.[9]

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So, our study was consistent with demonstrating the higher TOP2A expression protein, which was a poor predicting factor for luminal breast cancer, and this was consistent with the previous study.[11]

In our study, there is no relationship between the expression of TOP2A and age, tumor size, the status of lymph nodes, histological type, consistent with age, tumor size, the status of lymph nodes[11] and inconsistent with some studies which mentioned that the TOP2A protein has been frequently associated with node metastasis and tumor size.[10,14]

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Conflicts of interest
There are no conflicts of interest.

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