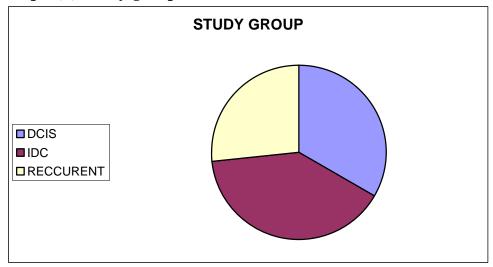
RESULTS

The studied cases include 10 cases of ductal carcinoma insitu, 20 cases of invasive ductal carcinoma (6 cases grade II and 6 cases of grade III) and 8 cases of recurrent invasive ductal carcinoma. Six cases of fibrocystic disease were taken as control cases (*Graph 1*):

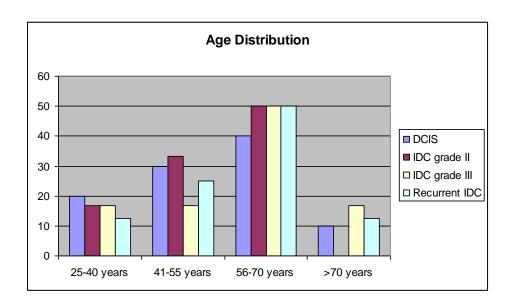
Graph (1):Study groups



Age distribution: The age of patients ranged from 28 to 76 years old (mean age 48 year, SD \pm 11.14). The highest age incidence was within the age group 56-70 years .The age distribution is shown in *Table 5 & Graph2*

Table (5): Age distribution in examined ductal carcinoma cases;

| Туре | No. of | 25 | -40 | >40-55 | | >55-70 | | >70 years | |
|---------------|--------|-----|------|--------|-------|--------|-------|-----------|------|
| | cases | ye | ars | years | | years | | | |
| | | No. | % | No | % | No. | % | No. | % |
| DCIS | 10 | 2 | 20 | 3 | 30 | 4 | 40 | 1 | 10 |
| IDC grade II | 6 | 1 | 16.7 | 2 | 33.3 | 3 | 50 | 0 | 0 |
| IDC grade III | 6 | 1 | 16.7 | 1 | 16.7 | 3 | 50 | 1 | 16.7 |
| Recurrent | 8 | 1 | 12.5 | 2 | 25 | 4 | 50 | 1 | 12.5 |
| | | | | | | | | | |
| Total | 30 | 5 | 16.7 | 8 | 26. 7 | 14 | 46. 7 | 3 | 10 |



Graph (2): Age distribution in examined cases of ductal carcinoma

Distribution of cases according to family history:

According to the family history of breast cancer, 4 cases (13.3%) had positive family history while 26 cases (86.7%) of all cases had no family history of breast cancer. The four cases of positive family history included one case of DCIS, two cases of IDC grade III and one case of recurrent ductal carcinoma.

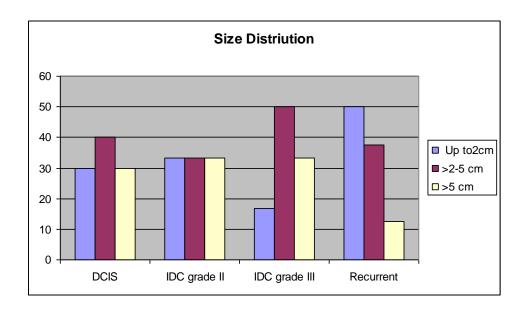
The size of the examined lesions:

The size of the examined breast carcinomas ranged from 0.5cm up to 13cm malignant masses divided according to the size into 3 groups: ten cases (33.3%) showed small masses (up to 2cm), 12 cases (40%) were with masses measuring >2-5 cm and 8 cases (26.7%) showed large masses (>5 cm in largest dimensions. The highest incidence of largest size was within the invasive carcinoma cases (*Table 6 and Graph 3*).

Table (6): Distribution of studied cases according to tumor size:

| Туре | No. of | Up t | o2cm | >2-5 | 5 cm | >5 cm | | |
|---------------|--------|------|------|------|------|-------|------|--|
| | cases | No. | % | No. | % | No. | % | |
| DCIS | 10 | 3 | 30 | 4 | 40 | 3 | 30 | |
| IDC grade II | 6 | 2 | 33.3 | 2 | 33.3 | 2 | 33.3 | |
| IDC grade III | 6 | 1 | 16.7 | 3 | 50 | 2 | 33.3 | |
| Recurrent | 8 | 4 | 50 | 3 | 37.5 | 1 | 12.5 | |
| Total | 30 | 10 | 33.3 | 12 | 40 | 8 | 26.7 | |

Graph (3): Distribution of cases according to size:



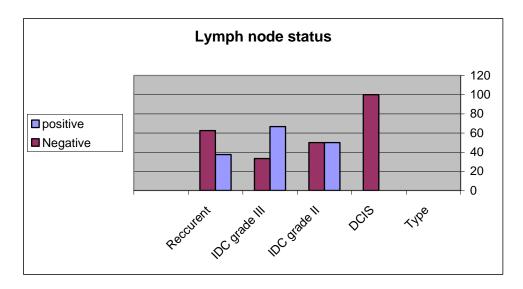
Lymph node status of the examined cases:

Regarding the Lymph node status, 10 cases (33.3%) were positive for lymph nodes metastasis while 20 cases (66.7%) of all cases show no lymph nodes metastasis. The higest incidence of lymph nodes metastasis was within cases of invasive ductal carcinoma grade III (table 7,graph 4).

Table (7): Distribution of cases according to lymph nodes metastasis

| | No. of | positive | e | Negative | | |
|---------------|--------|----------|------|----------|------|--|
| Туре | cases | No. | % | No. | % | |
| DCIS | 10 | 0 | 0 | 10 | 100 | |
| IDC grade II | 6 | 3 | 50 | 3 | 50 | |
| IDC grade III | 6 | 4 | 66.7 | 2 | 33.3 | |
| Reccurent | 8 | 3 | 37.5 | 5 | 62.5 | |
| Total | 30 | 10 | 33.3 | 20 | 66.7 | |

Graph (4): Distribution of cases according to lymph nodes metastasis



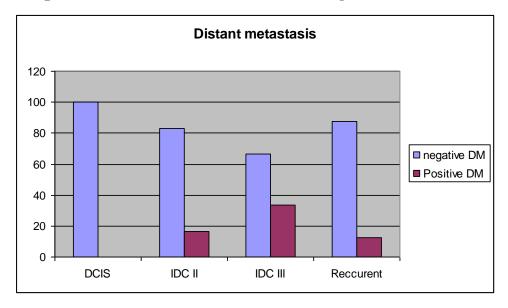
Distant metastasis of the examined cases:

Regarding distant metastasis, twenty six (86.7%) cases showed no distant metastasis while four cases(13.3%) showed positive distant metastasis. The highest incidence of distant metastasis was detected within invasive ductal carcinoma (table 8,graph 5).

Table (8): Distribution of cases according to distant metastasis

| Туре | No. of | negati | ve DM | Positi | ve DM |
|-----------|--------|--------|-------|--------|-------|
| | cases | No. | % | No. | % |
| DCIS | 10 | 10 | 100 | 0 | 0 |
| IDC II | 6 | 5 | 83.3 | 1 | 16.7 |
| IDC III | 6 | 4 | 66.7 | 2 | 33.3 |
| Reccurent | 8 | 7 | 87.5 | 1 | 12.5 |
| Total | 30 | 26 | 86.7 | 4 | 13.3 |

Graph (5) Distribution of cases according to distant metastasis



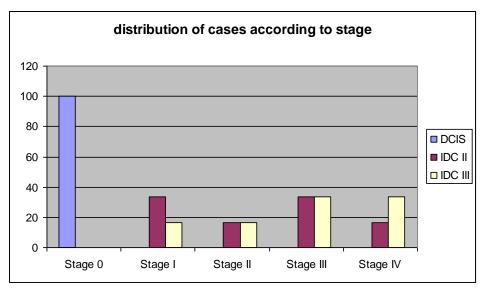
Staging of the examined cases:

According to TNM staging , ten cases were stage 0 (45.4 %) , three cases (13.6 %) were stage I ,two cases (9.1%) were stage II, four cases (18.2 %) were stage III, and three cases(13.6%) were stage IV.all cases of ductal carcinoma insitu were stage zero(**Table 9, Graph 6**)

Table (9): Distribution of cases according to the stage:

| Type | No. | | Stage | | | | | | | | | |
|---------|-----|-----|-------|-----|------|----|------|-----|------|-----|------|--|
| | | | 0 | | I | | II | | III | | IV | |
| | | No. | % | No. | % | No | % | No. | % | No. | % | |
| DCIS | 10 | 10 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| IDC II | 6 | 0 | 0 | 2 | 33,3 | 1 | 16,7 | 2 | 33,3 | 1 | 16,7 | |
| IDC III | 6 | 0 | 0 | 1 | 16,7 | 1 | 16,7 | 2 | 33,3 | 2 | 33,3 | |
| Total | 22 | 10 | 45.4 | 3 | 13.6 | 2 | 9.1 | 4 | 18.2 | 3 | 13.6 | |

Graph (6): Distribution of cases according to the stage:



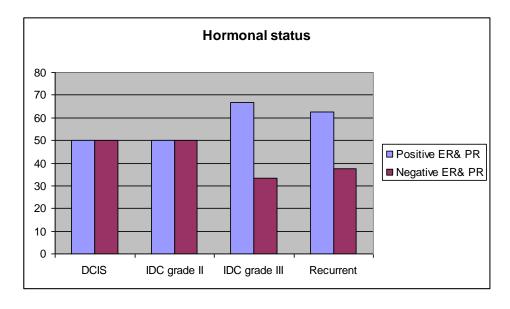
Hormonal status of the examined cases:

Regarding the hormonal status,17 cases (56.7%) were positive for ER and PR while 13 cases (43.3%) showed negative ER and PR. The positive ER and PR cases were mainly invasive ductal carcinoma cases(*table 10,graph 7*).

Table (10): Distribution of cases according to the hormonal status

| Туре | No. of | Pos | itive | Neg | ative |
|---------------|--------|--------|-------|-----|-------|
| | cases | ER | & PR | ER8 | k PR |
| | | No. % | | No. | % |
| DCIS | 10 | 5 50 | | 5 | 50 |
| IDC grade II | 6 | 3 | 50 | 3 | 50 |
| IDC grade III | 6 | 4 | 66.7 | 2 | 33.3 |
| Recurrent | 8 | 5 62.5 | | 3 | 37.5 |
| Total | 30 | 17 | 56.7 | 13 | 43.3 |

Graph (7): Distribution of cases according to the hormonal status



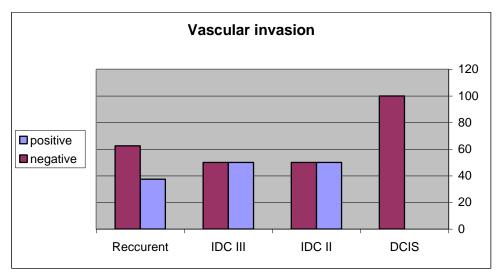
Vascular invasion of the examined cases:

Examination of vascular invasion of the cases revealed that 9 cases (36.7%) showed vascular invasion while 21 cases(63.3%) were free of vascular invasion. All cases of ductal carcinoma insitu were negative for vascular invasion. The highest incidence of vascular invasion was within invasive ductal carcinoma grade II and III (table11& graph 8)

Table (11): Distribution of cases according to vascular invasion

| type | No. of | +1 | ve | -ve | | |
|-----------|--------|-----|------|-----|------|--|
| | cases | No. | % | No. | % | |
| DCIS | 10 | 0 | 0 | 10 | 100 | |
| IDC II | 6 | 3 | 50 | 3 | 50 | |
| IDC III | 6 | 3 | 50 | 3 | 50 | |
| Reccurent | 8 | 3 | 37.5 | 5 | 62.5 | |
| Total | 30 | 9 | 36.7 | 21 | 63.3 | |

Graph (8): Distribution of cases according to vascular invasion



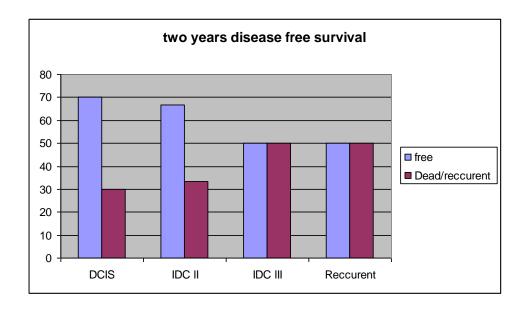
Two years survival of the examined cases:

According to two years survival, 18 cases (60 %) showed disease free survival while 12 cases(40%) showed poor survival as death or reccurence or second tissue reccurence. Cases which showed disease free survival were mainly ductal carcinoma insitu but cases that showed poor survival were mainly recurrent cases.and IDC grade III.(table12& graph 9)

Table (12): Distribution of cases according to two years suvival

| Туре | No. of | fr | ee | Dead/reccurent | | |
|-----------|--------|-----|-------|----------------|------|--|
| | cases | No. | No. % | | % | |
| DCIS | 10 | 7 | 70 | 3 | 30 | |
| IDC II | 6 | 4 | 66.7 | 2 | 33.3 | |
| IDC III | 6 | 3 | 50 | 3 | 50 | |
| Reccurent | 8 | 4 | 50 | 4 | 50 | |
| Total | 30 | 18 | 60 | 12 | 40 | |

Graph (9): Distribution of cases according to two years survival





 $Fig(11) \ \ Ductal \ carcinoma \ insitu, comedo \ pattern \ showing \ large, \ pleomorphic \ cellular \ lining \ which is associated \ with \ necrosis \ (H\&Ex100)$

Fig(12) Ductal carcinoma insitu, solid pattern with focal comedo The duct is completely obliterated by relatively small monotonous neoplastic cells (H&Ex100)



 $Fig(13) \ {\tt Ductal\ carcinoma\ insitu}, papillary\ pattern}(H\&Ex100)$

 $Fig(14) \ \ \text{Ductal carcinoma insitu, cribriform pattern} (\text{H\&Ex100})$



 $Fig(15) {\rm IDC~, grade~II~Malignant~cells~are~arranged~in~tubular~structures~with~relatively~nuclear~pleomorphism~(H\&Ex200)}$

Fig(16) Invasive Ductal carcinoma ,grade II, muscular invasion(H&Ex100)



 $Fig(17) \hbox{Invasive Ductal carcinoma grade III showing solid sheets of malignant cells with no tubular formation } (H\&EX200)$

Fig(18)Invasive Ductal carcinoma grade III, reccurent, showing solid sheets of malignant cells with no tubular formation and cellular pleomorphism (**H&EX400**)



 $Fig(19) \ {\bf Invasive} \ \ {\bf Ductal} \ \ {\bf carcinoma} \ \ , \\ {\bf grade} \ \ {\bf III} \ \ {\bf with} \ \ {\bf vascular} \ \ {\bf emboli} ({\bf H\&EX200})$

 $Fig(20) \ \ {\bf Invasive} \ \ {\bf Ductal\ carcinoma\ }, Grade\ III, reccurent\ with\ fat\ invasion(H\&EX200)$

Immunohistochemical results of CD44⁺/CD24^{-/low} breast Cancer stem cells

Cancer stem cells in study groups

Out of 10 cases of DCIS, five cases(50%) showed +1 breast cancer stem cells(BCSCs) and one case(10%) showed +2 BCSCs while four cases (40%)showed zero (CD44+ve CD24-ve/low) BCSCs

Out of 6 cases of IDC grade II ,one case(16.7%) showed +1 BCSCs, 1case (16.7%)showed +2 BCSCs, and 1case (16.7%)showed +3 BCSCs while three cases(50%) showed zero BCSCs,.

Out of 6 cases of IDC grade III ,three cases(50%) showed +1 BCSCs and one case (16.7%)showed +3 BCSCs while two cases (33.3%)showed zero BCSCs

Out of 8 reccurent cases, one cases(12.5%) showed +1 BCSCs, five cases (62.5) showed +2 BCSCs and two cases (25%) showed +3 BCSCs. (Table 13).

The distribution of BCSCs was variable in different sections From focal ,segmental to diffuse staining and also variable in intensity from strong to week membranous staining.

As regard to control cases ,no CD44⁺/CD24^{-/low} breast cancer stem cells were detected in six cases of fibrocystic disease but only focal CD24 staining (brown membranous staining) was detected.

| STUDY GROUP | No. | | CD44 ⁺ /CD24 ^{-/low} breast Cancer stem cells | | | | | | | | | |
|----------------|-----|----|---|---|------|---|------|---|------|--|--|--|
| | | | 0 +1 +2 +3 | | | | | | | | | |
| | | No | | | | | | | | | | |
| DCIS | 10 | 4 | 40 | 5 | 50 | 1 | 10 | 0 | 0 | | | |
| IDC II | 6 | 3 | 50 | 1 | 16.7 | 1 | 16.7 | 1 | 16.7 | | | |
| IDC III | 6 | 2 | 2 33.3 3 50 0 0 1 16.7 | | | | | | | | | |
| Recurrent | 8 | 0 | 0 | 1 | 12.5 | 5 | 62.5 | 2 | 25 | | | |

Table (13): Distribution of Cancer stem cells in study groups

1- correlation between cancer stem cells and age of patient

10

30

30

Total

- Out of 5 cases of age group(25-40 years), one case(10%) showed +1 BCSCs, and one case(10%) showed +3 BCSCs while three cases (30%) showed zero BCSCs,

33.3

7

23.3

13.3

- Out of 8 cases of age group(41-55 years), five cases (62.5%)showed +1 BCSCs and two cases (25%)showed +2 BCSCs while 0ne case(12.5%) showed zero BCSCs,
- Out of 14 cases of age group(56-70 years), three cases(21.4%) showed +1 BCSCs, three cases (21.4%) showed +2 BCSCs and three (21.4%) cases showed +3 BCSCs while five cases(35.7%) showed zero BCSCs
- Out of 3 cases of age group(71-80 years), one case (33.3%)showed +1 BCSCs and two cases(66.7%) showed +2 BCSCs,

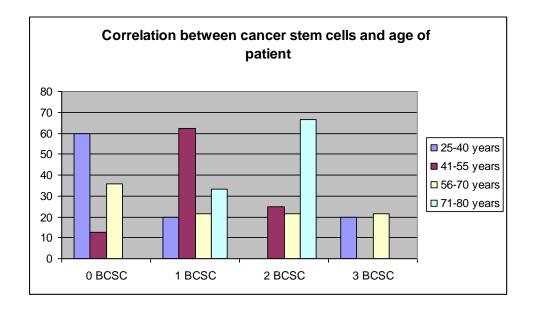
There was no statistically significant correlation between BCSCs expression and the age of patient (p=0,236).

This is illustrated in table (14) and graph (10)

Table (14):_Correlation between cancer stem cells and age of patient

| Age | No | | b | | 044 ⁺ /C Canc | | | ls | |
|---------------|----|----|------|-----|-----------------------------|----|------|-----|------|
| | | (| 0 | + | -1 | 4 | -2 | + | -3 |
| | | No | % | No. | % | No | % | No. | % |
| 25-40 years | 5 | 3 | 60 | 1 | 20 | 0 | 0 | 1 | 20 |
| 41-55 years | 8 | 1 | 12,5 | 5 | 62.5 | 2 | 25 | 0 | 0 |
| 56-70 years | 14 | 5 | 35,7 | 3 | 21,4 | 3 | 21.4 | 3 | 21.4 |
| 71-80 years | 3 | 0 | 0 | 1 | 33.3 | 2 | 66.7 | 0 | 0 |
| Total | 30 | 9 | 30 | 10 | 33.3 | 7 | 23.3 | 4 | 13.3 |
| P value=0,236 | | | | | | | | | |

Graph (10): Correlation between cancer stem cells and age of patient



2- Correlation between cancer stem cells and size of the tumor

-Out of 10 cases of size group(up to 2 cm), one case(10%) showed +1 BCSCs, four cases (40%) showed +2 BCSCs and one case (10%) showed +3 BCSCs while four cases(40%) showed zero BCSCs

-Out of 12 cases of size group(>2- 5 cm), five cases(**41.7**%) showed +1 BCSCs, two cases (**16.7**%) showed +2 BCSCs and two cases (**16.7**%) showed +3 BCSCs while three cases(25%) showed zero BCSCs

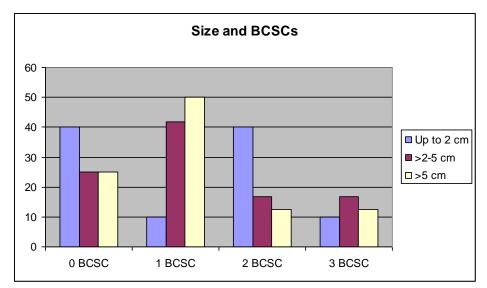
-Out of 8 cases of size group(> 5 cm), four cases (50%)showed +1 BCSCs, one case (12.5%)showed +2 BCSCs and one case(12.5%) showed +3 BCSCs while two cases (25%) showed zero BCSCs.

There was no statistically significant correlation between BCSCs expression and the size of the tumor(p=0.893).

This is illustrated in table (15) and graph(11)

Table (15): Correlation between cancer stem cells and size of the tumor

| Size | No | | b | | 044 ⁺ /C Canc | | | ls | | | | |
|---------------|----|----|------------|----|-----------------------------|---|------|----|------|--|--|--|
| 220 | | | 0 +1 +2 +3 | | | | | | | | | |
| | | No | | | | | | | | | | |
| Up to 2 cm | 10 | 4 | 40 | 1 | 10 | 4 | 40 | 1 | 10 | | | |
| >2-5 cm | 12 | 3 | 25 | 5 | 41.7 | 2 | 16.7 | 2 | 16.7 | | | |
| >5 cm | 8 | 2 | 25 | 4 | 50 | 1 | 12.5 | 1 | 12.5 | | | |
| Total | 30 | 9 | 30 | 10 | 33.3 | 7 | 23.3 | 4 | 13.3 | | | |
| P value=0.893 | | | | | | | | | | | | |



graph (11): Correlation between cancer stem cells and size of the tumor

3-Correlation between cancer stem cells and family history.

-Out of 4 cases having positive family history ,2 cases(50%) showed +1 BCSCs and one case (25%) showed +3 BCSCs while one case (25%)showed zero BCSCs.

-Out of 26 cases having negative family history, eight cases(**30.7**%) showed +1 BCSCs, seven (26.9%) cases showed +2 BCSCs and three cases (11.5%) showed +3 BCSCs while eight cases (30.7%)showed zero BCSCs.

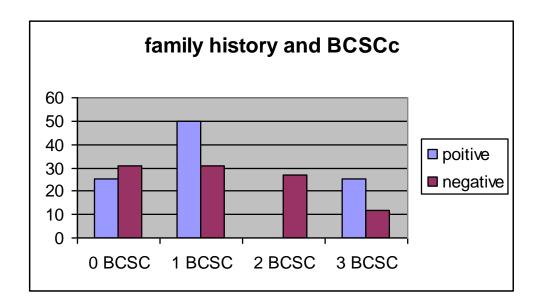
There was no statistically significant correlation between BCSCs expression and the family history (p=0.919)

This is illustrated in table (16) and figure (12)

Table (16): Correlation between cancer stem cells and family history

| FAMILY HISTORY | No | | CD44 ⁺ /CD24 ^{-/low} breast Cancer stem cells | | | | | | | | | |
|-------------------|----|----|---|----|------|---|------|---|------|--|--|--|
| | | | 0 +1 +2 +3 | | | | | | | | | |
| | | No | No % NO % NO % No. % | | | | | | | | | |
| Positive | 4 | 1 | 25 | 2 | 50 | 0 | 0 | 1 | 25 | | | |
| Negative | 26 | 8 | 30.7 | 8 | 30.7 | 7 | 26.9 | 3 | 11.5 | | | |
| Total | 30 | 9 | 30 | 10 | 33.7 | 7 | 23.3 | 4 | 13.3 | | | |
| P value=0.919 | | | | | | | | | | | | |

Graph (12): Correlation between cancer stem cells and family history



4-Correlation between cancer stem cells and type of ductal carcinoma

-Out of 10 cases of DCIS, five cases (50-%) showed +1 BCSCs and one case (10%)showed +2 BCSCs while four cases (40%) showed zero BCSCs

-Out of 20 cases of IDC ,five cases (25%)showed +1 BCSCs, six cases (30%) showed +2 BCSCs and four cases (20 %)showed +3 BCSCs while five cases (25%) showed zero BCSCs

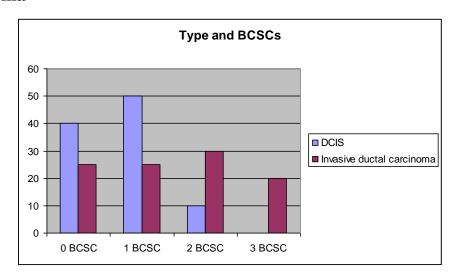
There was no statistically significant correlation between BCSCs expression and the type of ductal carcinoma (p=0,066).

This is illustrated in table (17) and graph (13).

Table (17): Correlation between cancer stem cells and type of ductal carcinoma

| Туре | No | | CD44 ⁺ /CD24 ^{-/low} breast Cancer stem cells | | | | | | | | | |
|---------------------------|----|----|---|----|------|----|------|------|------|--|--|--|
| | | | 0 | | +1 | | +2 | | -3 | | | |
| | | No | % | NO | % | NO | % | No · | % | | | |
| DCIS | 10 | 4 | 40 | 5 | 50 | 1 | 10 | 0 | 0 | | | |
| Invasive ductal carcinoma | 20 | 5 | 25 | 5 | 25 | 6 | 30 | 4 | 20 | | | |
| Total | 30 | 9 | 30 | 10 | 33.3 | 7 | 23.3 | 4 | 13.3 | | | |
| P value=0,066 | | | | | | | | | | | | |

graph (13): Correlation between cancer stem cells and type of ductal carcinoma



5-Correlation between cancer stem cells and grading

-Out of 15 cases of ductal carcinoma grade II, four cases(26.7%) showed +1 BCSCs, three cases(20%) showed +2 BCSCs and three cases(20%) showed +3 BCSCs while five cases(33.3%) showed zero BCSCs.

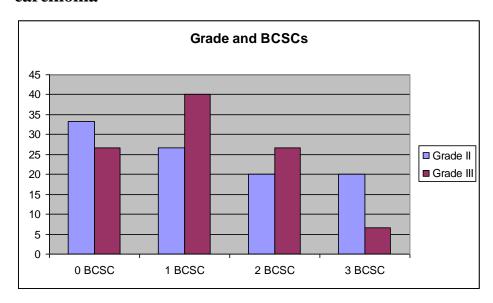
-Out of 15 cases of ductal carcinoma grade III ,six cases(40%) showed +1 BCSCs, four cases (26.7%) showed +2 BCSCs,and one case (6.7%)showed +3 BCSCs while four cases (26.7%)showed zero BCSCs

There was no statistically significant correlation between BCSCs expression and the grading of ductal carcinoma (p=0,730). This is illustrated in table (18) and graph (14).

Table (18):_Correlation between cancer stem cells and grading of ductal carcinoma

| GRADE | No | CD44 ⁺ /CD24 ^{-/low} breast Cancer stem cells | | | | | | | | | |
|---------------|----|---|------|----|------|----|------|-----|------|--|--|
| GRIDE | | 0 | | +1 | | +2 | | +3 | | | |
| | | No | % | NO | % | NO | % | No. | % | | |
| II | 15 | 5 | 33.3 | 4 | 20 | 3 | 20 | | | | |
| III | 15 | 4 | 26.7 | 6 | 40 | 4 | 26.7 | 1 | 6.7 | | |
| Total | 30 | 9 | 30 | 10 | 33.3 | 7 | 23.3 | 4 | 13.3 | | |
| P value=0,730 | | | | | • | | | | | | |

graph (14): Correlation between cancer stem cells and grading of ductal carcinoma



6 -Correlation between cancer stem cells in primary and recurrent cases

-All 8 reccurant cases(100%) were positive fo BCSCs, one case (12.5) showed +1 BCSCs, five cases (26.5) showed +2 BCSCs and two (25%)cases showed +3 BCSCs.

-Out of 22 non reccurant cases, nine cases(40.9%) showed +1 BCSCs, two cases (9.1%) showed +2 BCSCs and two cases (9.1%) showed +3 BCSCs while nine cases(40.9%) showed zero BCSCs

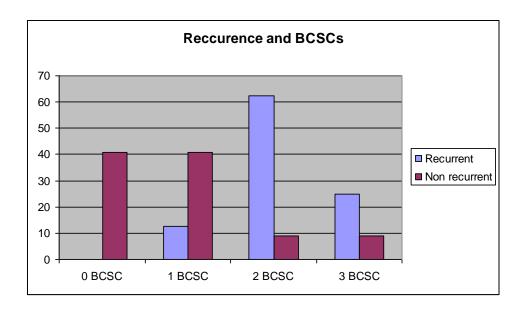
There was statistically significant correlation between BCSCs expression and the recurrence (p<0,05)

This is illustrated in table (19) and figure (15)

Table (19): Correlation between cancer stem cells primary and recurrent cases

| Reccurance | No | | CD44 ⁺ /CD24 ^{-/low} breast Cancer stem cells | | | | | | | | | |
|----------------|----|---------|---|----|------|----|------------|-----|-----|--|--|--|
| Trecedi unice | | 0 +1 +2 | | | | | ⊦ 2 | +3 | | | | |
| | | No | % | NO | % | NO | % | No. | % | | | |
| Recurrent | 8 | 0 | 0 | 1 | 12.5 | 5 | 62,5 | 2 | 25 | | | |
| Non recurrent | 22 | 9 | 9 40.9 9 40.9 2 9,1 2 | | | | | | 9.1 | | | |
| Total | 30 | 9 | 9 30 10 33.3 7 23,3 4 13. | | | | | | | | | |
| P value p<0,05 | | | | | | | | | | | | |

graph (15): Correlation between cancer stem cells primary and recurrent cases



7- Correlation between cancer stem cells and lymph nodes metastasis

-Out of 10 cases having positive LN s metastasis, five cases (50 %) showed +1 BCSCs, one case (10%) showed +2 BCSCs and three cases (30%) showed +3 BCSCs while one case (10%) showed zero BCSCs,

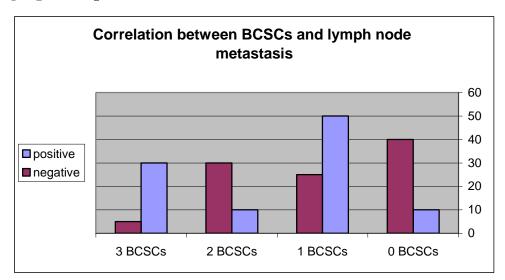
- Out of 20 cases having negative LN s metastasis, five cases (25%) showed +1 BCSCs, six cases (30%) showed +2 BCSCs and one case (5%) showed +3 BCSCs while eight cases (40%) showed zero BCSCs

There was statistically significant correlation between BCSCs expression and the lymph nodes metastasis (p=0.135)

This is illustrated in table (20) and figure (16)

Table (20): Correlation between cancer stem cells and LN metastasis

| LN | No | | b | | 044 ⁺ /C Canc | | | ls | | | |
|---------------|----|----|--|---|-----------------------------|---|----|----|----|--|--|
| | | | 0 +1 +2 +3 No % NO % No. % | | | | | | | | |
| | | No | | | | | | | | | |
| positive | 10 | 1 | 10 | 5 | 50 | 1 | 10 | 3 | 30 | | |
| negative | 20 | 8 | 40 | 5 | 25 | 6 | 30 | 1 | 5 | | |
| Total | 30 | 9 | 9 30 10 33.3 7 23.3 4 13.3 | | | | | | | | |
| P value=0.135 | | | | | | | | | | | |



graph(16): Correlation between cancer stem cells and LN metastasis

8- Correlation between cancer stem cells and distant metastasis

- Out of 4 cases having positive distant metastasis, one case (25%)showed +1 BCSCs, one case(25%) showed +2 BCSCs and two cases (50%) showed +3 BCSCs.

-Out of 26 cases having negative distant metastasis ,nine cases(34.6%) showed +1 BCSCs, six cases(23.1%) showed +2 BCSCs, and two cases(7.7%) showed +3 BCSCs while nine cases(34.6%) showed zero BCSCs

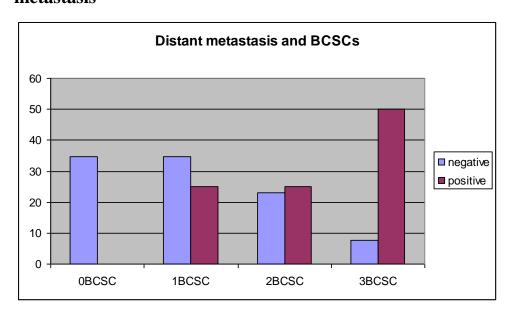
There was statistically significant correlation between BCSCs expression and the lymph nodes metastasis (p<0.05)

This is illustrated in table (21) and graph (17)

Table (21): Correlation between cancer stem cells and distant metastasis

| Distant metastasis | No | CD44 ⁺ /CD24 ^{-/low} breast Cancer stem cells | | | | | | | | | | | |
|-----------------------|----|--|--|----|------|---|------|---|------|--|---|--|----|
| | | | 0 +1 +2 +3 No % NO % No. % | | | | | | | | 0 | | -3 |
| | | No | | | | | | | | | | | |
| negative | 26 | 9 | 34.6 | 9 | 34.6 | 6 | 23.1 | 2 | 7.7 | | | | |
| positive | 4 | 0 | 0 | 1 | 25 | 1 | 25 | 2 | 50 | | | | |
| Total | 30 | 9 | 30 | 10 | 33.3 | 7 | 23.3 | 4 | 13.3 | | | | |
| P value<0,05 | | | | | | | | | | | | | |

graph (17): Correlation between cancer stem cells and distant metastasis



9- Correlation between cancer stem cells and stage of tumor

- Out of ten cases with stage 0, five cases(50%) showed +1 BCSCs and one case (10%) showed +2 BCSCs while four cases (40%)showed zero BCSCs,

- Out of three cases with stage I, one case(16.7%) showed +2 BCSCs while two cases(33.3%) showed zero BCSCs
 - Out of two cases with stage II, two cases(100%) showed zero BCSCs.
- Out of four cases with stage III, one case (25%)showed zero BCSCs while three cases (75%) showed +1 BCSCs,

Out of three cases with stage IV, one case(25%) showed +1 BCSCs and two cases (75%) showed +3 BCSCs.

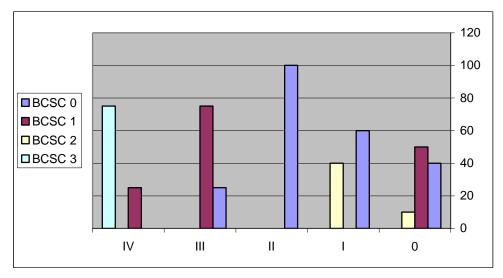
There was no statistically significant correlation between BCSCs expression and the stage (p=0.068)

This is illustrated in table (22) and figure (18)

Table (22): Correlation between cancer stem cells and stage of the tumor

| | | CD44 ⁺ /CD24 ^{-/low} breast Cancer stem cells | | | | | | | | | |
|---------------|----|---|------|-----|------|----|------|-----|------|--|--|
| STAGE | No | | 0 | +1 | | - | +2 | +3 | | | |
| | | No | % | No. | % | No | % | No. | % | | |
| 0 | 10 | 4 | 40 | 5 | 50 | 1 | 10 | 0 | 0 | | |
| I | 3 | 2 | 60 | 0 | 0 | 1 | 40 | 0 | 0 | | |
| II | 2 | 2 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| III | 4 | 1 | 25 | 3 | 75 | 0 | 0 | 0 | 0 | | |
| IV | 3 | 0 | 0 | 1 | 25 | 0 | 0 | 2 | 75 | | |
| Total | 22 | 9 | 33.3 | 9 | 33.3 | 2 | 14.8 | 2 | 14.8 | | |
| P value=0.068 | | | | | | | | | | | |

graph (18):_Correlation between cancer stem cells and stage of the tumor



10- Correlation between cancer stem cells and hormonal status.

Out of 17 cases having positive estrogen and progesterone receptors ,five cases(29.4%) showed +1 BCSCs, three cases (17.6%)showed +2 BCSCs and one case (5.9%)showed +3 BCSCs while eight cases (47%)showed zero BCSCs.

Out of 13 cases having negative estrogen and progesterone receptors ,five cases (38.4%)showed +1 BCSCs, four cases(30.8%) showed +2 BCSCs and three cases (23.1%) showed +3 BCSCs while one case (7.7%)showed zero BCSCs,

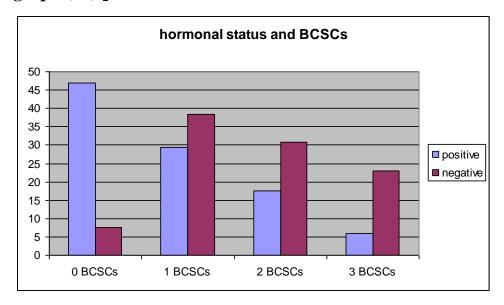
There was statistically significant correlation between BCSCs expression and the hormonal status (p<0,05)

.This is illustrated in table (23) and figure (19)

Table (23): Correlation between cancer stem cells and hormonal status

| Hormonal | No | | b | | 044 ⁺ /C Canc | | | ls | |
|----------------------|----|----|--------------------|----|-----------------------------|----|------|-----|------|
| status | | | 0 +1 | | +2 | | + | -3 | |
| | | No | % | NO | % | NO | % | No. | % |
| positive | 17 | 8 | 8 47 5 29.4 3 17.6 | | | | | | |
| negative | 13 | 1 | 7.7 | 5 | 38.4 | 4 | 30.8 | 3 | 23.1 |
| Total | 30 | 9 | 30 | 10 | 33.3 | 7 | 23.3 | 4 | 13.3 |
| <i>P value</i> <0,05 | | | | | | | | | |

graph (19): Correlation between cancer stem cells and hormonal status



11-Correlation between cancer stem cells and vascular invasion.

Out of 9 cases having positive vascular invasion, two cases (22.2%)showed +1 BCSCs, one case(11.1%) showed +2 BCSCs and four cases(44.4%) showed +3 BCSCs while two cases(22.2%) showed zero BCSCs

Out of 21 cases having negative vascular invasions, eight cases (38.1%) showed +1 BCSCs, six cases (28.8%) showed +2 BCSCs while seven cases (33.3%) showed zero BCSCs.

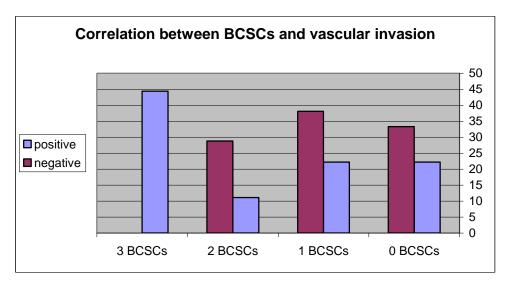
There was statistically significant correlation between BCSCs expression and the vascular invasion (p < 0.05)

This is illustrated in table (24) and graph (20)

Table (24): Correlation between cancer stem cells and vascular invasion

| Vascular | No | | CD44 ⁺ /CD24 ^{-/low} breast Cancer stem cells | | | | | | | | | | |
|----------------------|----|----|---|----|------|----|------|-----|------|--|--|--|--|
| invasion | | 0 | | +1 | | +2 | | +3 | | | | | |
| | | No | % | NO | % | NO | % | No. | % | | | | |
| positive | 9 | 2 | 22.2 | 2 | 22.2 | 1 | 11.1 | 4 | 44.4 | | | | |
| negative | 21 | 7 | 33.3 | 8 | 38.1 | 6 | 28.8 | 0 | 0 | | | | |
| Total | 30 | 9 | 30 | 10 | 33.3 | 7 | 23.3 | 4 | 13.3 | | | | |
| <i>P value</i> <0,05 | | | | | | | | | | | | | |

graph (20): Correlation between cancer stem cells and vascular invasion



12 -correlation between cancer stem cells and two years survival

Out of 18 cases having disease free survival ,five cases (27.8%) showed +1 BCSCc, four cases(22.2%) showed +2 BCSCs and one case(5.5%) showed +3 BCSCs while eight cases (44.4%)showed zero BCSCs,

Out of 12 cases with poor survival, five cases(41.7%) showed +1 BCSCc, three cases(25%) showed +2 BCSCs, three cases(25%) showed +3 BCSCs while one case (8.3%)showed zero BCSCs.

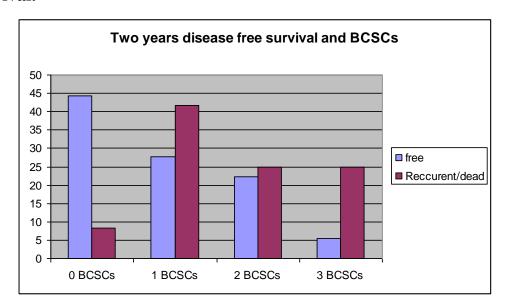
There was statistically significant correlation between BCSCs expression and two years survival (p<0.05)

This is illustrated in table (25) and graph (21)

Table (25):_Correlation between cancer stem cells and two years survival

| Two years | No | | CD44 ⁺ /CD24 ^{-/low} breast Cancer stem cells | | | | | | | | | |
|----------------------|----|---------|---|----|------|----|------|-----|-----|--|--|--|
| survival | | 0 +1 +2 | | | | | | | -3 | | | |
| | | No | % | NO | % | NO | % | No. | % | | | |
| free | 18 | 8 | 44.4 | 5 | 27.8 | 4 | 22.2 | 1 | 5.5 | | | |
| Reccurent/dead | 12 | 1 | 1 8.3 5 41.7 3 25 3 | | | | | | | | | |
| Total | 30 | 9 | 9 30 10 33.3 7 23.3 4 13.3 | | | | | | | | | |
| <i>P value</i> <0,05 | | | | | | | | | | | | |

graph (21):_Correlation between cancer stem cells and two years survival.



 $Table\ (26): \underline{\ } Correlation\ between\ cancer\ stem\ cells\ and\ Histopathological\ variants:$

| Histopathological variants | | В | reast cand | er stem cel | ls | |
|----------------------------|----------------|-------|------------|-------------|-------|--------------------------|
| | | zero | +1 | +2 | +3 | P Value |
| Age | 25-40 years | 60% | 20% | 0% | 20% | p=0,236. |
| | 41-55 years | 12,5% | 62.5% | 25% | 0% | insignifican t |
| | 56-70 years | 35,7% | 21,4% | 21.4% | 21.4% | |
| | >70 years | 0% | 33.3% | 66.7% | 0% | |
| Size | Up to 2 cm | 40% | 10% | 40% | 10% | p=0.893 |
| | >2-5 cm | 25% | 41.7 | 16.7% | 16.7% | insignificant |
| | > 5 cm | 25% | 50% | 12.5% | 12.5% | |
| | negative | 30.7% | 30.7% | 26.9% | 11.5% | |
| Type | DCIS | 40% | 50% | 10% | 0% | p=0,066 |
| | IDC | 25% | 25% | 30% | 20% | - insignificant |
| Grade | Grade II | 33.3% | 26.7% | 20% | 20% | p=0,730 insignificant |
| | Grade III | 26.7% | 40% | 26.7% | 6.7% | insignijičani |
| Lymph nodes metastasis | Positive | 10% | 50% | 10% | 30% | p=0.135 |
| metastasis | negative | 40% | 30% | 25% | 5% | insignificant |
| Distant metastasis | negative | 34.6% | 34.6% | 23.1% | 7.7% | p<0,05 significant |
| metastasis | Positive | 0% | 25% | 25% | 50% | signijicani |
| stage | 0 | 40% | 50% | 10% | 0% | |
| | I | 60% | 0% | 40% | 0% | p=.068 |
| | II | 100% | 0% | 0% | 0% | Insignificant |
| | III | 25% | 75% | 0% | 0% | |
| | IV | 0% | 25% | 0% | 75% | |
| reccurence | reccurent | 0% | 12.5% | 62,5% | 25% | p<0,05 |
| | Non reccurent | 40.9% | 40.9% | 9,1% | 9.1% | - significant |
| Hormonal | Positive | 47% | 29.4% | 17.6% | 5.9% | p<0,05 |
| statue | negative | 7.7% | 38.4% | 30.8% | 23.1% | - significant |
| Vascular | Positive | 22.2% | 22.2% | 11.1% | 44.4% | p<0,05 |
| invasion | negative | 33.3% | 38.1% | 28.8% | 0% | significant |
| Two years disease free | free | 44.4% | 27.8% | 22.2% | 5.5% | p<0,05 significant |
| survival | Dead/reccurent | 8.3% | 41.7% | 25% | 25% | |





 $Fig(23) \hbox{Invasive ductal carcinoma }, positive for CD44+CD24+cells \ . \ BCSCs \ scoring \ zero(double immunohistochemical techniquex400)$

 $Fig(24) \ \, {\rm Ductal\ carcinoma\ insitu\ , positive\ for\ CD44-CD24+cells,\ .\ BCSCs\ scoring\ zero(double\ immunohistochemical\ techniquex400)}$



 $Fig(25) \ \ {\rm Invasive} \ \ {\rm ductal} \ \ {\rm carcinoma} \ \ , \ positive \ \ {\rm for} \ \ {\rm CD44-CD24+cells} \ . \ \ BCSCs \ scoring \ zero(double immunohistochemical techniquex400)$

Fig(26) Invasive ductal carcinoma, positive for CD44-CD44+cells and CD44+CD24+ cells, BCSCs, scoring zero(double immunohistochemical techniquex400).



 $Fig(27) \ \, {\rm Ductal\ carcinoma\ insitu}, positive\ for\ CD44-CD24-\ cells\ (A)\ CD44^+/CD24^{-/low}\ BCSCs\ (B) scoring\ +1 (double\ immunohistochemical\ techniquex400)$

Fig(28)) Invasive ductal carcinoma positive for CD44 $^{\!\!\!\!\!\!^{+}}\!/\text{CD24}^{\!\!\!\!\!^{-\!\!\!\!\!\!\!\!/}\!\text{ODW}}$ BCSCs scoring +1 (double immunohistochemical techniquex400)



 $Fig(29) \ \ \ Invasive \ ductal \ carcinoma \ \ , positive \ for \ CD44^+/CD24^{-/low} \ BCSCs \ \ scoring \ +1 \ (double \ immunohistochemical \ techniquex1000)$

 $Fig(30) \ \, {\rm Ductal\ carcinoma\ insitu}, \ positive\ for\ CD44^+/CD24^{-{\rm flow}}\ BCSCs\ \ scoring\ +2\ (double\ immunohistochemical\ techniquex400)$



 $Fig(31) \ \ \ \ Invasive ductal \ carcinoma \ positive \ for \ CD44^+/CD24^{-/low} \ BCSCs \ scoring \ +2 \ (double \ immunohistochemical \ techniquex400)$

 $Fig(32) \ \ \text{Invasive ductal carcinoma , recurrent, positive for CD44$^+/CD24$^--low BCSCs scoring $+2$ (double immunohistochemical techniquex400)}$



Fig(33) Invasive ductal carcinoma , positive for CD44⁺/CD24^{-/low} BCSCs scoring +3 (double immunohistochemical techniquex200)

 $Fig(34) \ \ \ \text{Invasive ductal carcinoma , positive for CD44$^+/CD24$^-/low BCSCs scoring +3 (double immunohistochemical techniquex400)}$