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***Retrospective Study***

**Forkhead box P3 and indoleamine 2,3-dioxygenase co-expression in Pakistani triple negative breast cancer patients**

Asghar K *et al*. FOXP3 and IDO

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**Abstract**

BACKGROUND

Forkhead box P3 (FOXP3) is a specific marker for immunosuppressive regulatory T (T-reg) cells. T-regs and an immunosuppressive enzyme, indoleamine 2,3-dioxygenase (IDO), are associated with advanced disease in cancer.

AIM

To evaluate the co-expression of FOXP3 and IDO in triple negative breast cancer (TNBC) with respect to hormone-positive breast cancer patients from Pakistan.

METHODS

Immunohistochemistry was performed to analyze the expression of FOXP3, IDO, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor on tissues of breast cancer patients (*n* = 100): Hormone-positive breast cancer (*n* = 51) and TNBC (*n* = 49). A total of 100 patients were characterized as FOXP3 negative *vs* positive and further categorized based on low, medium, and high IDO expression score. Univariate and multivariate logistic regression models were used.

RESULTS

Out of 100 breast tumors, 25% expressed FOXP3 positive T-regs. A significant co-expression of FOXP3 and IDO was observed among patients with TNBC (*P* = 0.01) compared to those with hormone-positive breast cancer. Two variables were identified as significant independent risk factors for FOXP3 positive: IDO expression high (adjusted odds ratio (AOR) 5.90; 95% confidence interval (CI): 1.22-28.64; *P* = 0.03) and TNBC (AOR 2.80; 95% CI: 0.96-7.95; *P* = 0.05).

CONCLUSION

Our data showed that FOXP3 positive cells might be associated with high expression of IDO in TNBC patients. FOXP3 and IDO co-expression may also suggest its involvement in disease, and evaluation of FOXP3 and IDO expression in TNBC patients may offer a new therapeutic option.

**Key Words:** Forkhead box P3; Indoleamine 2,3-dioxygenase; Triple negative breast cancer; T-regs; Immunotherapy; Cancer

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**Core Tip:** Forkhead box P3 (FOXP3) positive cells might be associated with high expression of indoleamine 2,3-dioxygenase (IDO) in triple negative breast cancer (TNBC) patients. Evaluation of FOXP3 and IDO expression in TNBC patients may provide a novel effective therapeutic strategy.

**INTRODUCTION**

Forkhead box P3 (FOXP3) is a part of the forkhead/winged-helix family of transcription regulators[1]. FOXP3 is a specific marker for regulatory T cells (T-regs)[[2](#_ENREF_2" \o "Curiel, 2008 #460)], which are crucial mediators of peripheral tolerance[[3](#_ENREF_3" \o "Watanabe, 2010 #461)]. FOXP3 expression has been reported in breast cancer[[4-6](#_ENREF_4" \o "Ladoire, 2011 #462)], and its quantification in this malignancy can be used as an effective tool to monitor disease progression and predict prognosis[[7](#_ENREF_7" \o "Bates, 2006 #465)]. The cell count of FOXP3 expressing T-regs increases steadily in breast cancer with increasing stage of disease[[7](#_ENREF_7" \o "Bates, 2006 #465)]. The mechanisms underlying are still not clear. High numbers of FOXP3 expressing T-regs provide poor prognosis for relapse-free survival in patients with invasive carcinoma[[7](#_ENREF_7" \o "Bates, 2006 #465)], but Lee *et al*[[8](#_ENREF_8" \o "Lee, 2013 #467)] observed the prognostic significance of FOXP3-positive T-regs compared to FOXP3-negative T-regs in triple negative breast cancer (TNBC). Furthermore, they found that improved survival was linked with FOXP3-postive T-regs in TNBC. This finding was in contrast with other types of cancers[[8](#_ENREF_8" \o "Lee, 2013 #467)]. Therefore, further studies are required to link FOXP3- positive T-regs to good or worse prognosis.

An immunosuppressive enzyme, indoleamine 2,3-dioxygenase (IDO), catabolizes tryptophan into kynurenines[[9](#_ENREF_9" \o "Mansfield, 2009 #468),[10](#_ENREF_10" \o "Muller, 2005 #469)]. IDO has the ability to inhibit the immune responses and produce immunosuppression through the differentiation and maturation of T-regs[[11](#_ENREF_11" \o "Mellor, 1999 #470)]. On the other hand, tryptophan depletion by IDO affects the cytotoxicity of T cells[[12](#_ENREF_12" \o "Lee, 2002 #472)]. It has been reported that tryptophan downstream metabolites induce apoptosis of T cells *in vitro*[[13](#_ENREF_13" \o "Munn, 1999 #474)]. IDO plays a role in the cancer immune-escape mechanism[[14](#_ENREF_14" \o "Mellor, 2004 #475),[15](#_ENREF_15" \o "Grohmann, 2003 #476)]. Evidence has suggested that overexpression of IDO has been observed in both antigen-presenting cells and tumor cells in tumor draining lymph nodes[[16](#_ENREF_16" \o "Katz, 2008 #477)]. IDO overexpression may lead to recruitment of T-regs in breast tumor microenvironment and promote metastasis[[17](#_ENREF_17" \o "Yu, 2011 #478)].

TNBC is characterized by lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER2)[[18](#_ENREF_18" \o "Curigliano, 2011 #482)]. TNBC is a more aggressive tumor than other breast cancers types[[19](#_ENREF_19" \o "Penault-Llorca, 2012 #484)]. Our goal was to quantify FOXP3 expression in relation with IDO expression in patients diagnosed with breast cancer from Pakistan. Pakistan has the highest incidence of breast cancer cases in its region. We further investigated the numbers of FOXP3-positive T-regs in TNBC patients compared to hormone-positive breast cancer patients.

**MATERIALS AND METHODS**

***Sampling and patient data***

For this retrospective analysis, archived formalin-fixed paraffin-embedded (FFPE) blocks of 100 breast cancer patients were retrieved from the pathology department. The study was conducted at Shaukat Khanum Memorial Cancer Hospital and Research Center (SKMCH&RC) Lahore, Pakistan. All the patients were diagnosed with breast cancer between 2007 and 2009, and all patients selected were treatment naïve. Tumor grade was allocated using the Nottingham Histologic Score. Immunohistochemistry was performed to identify the expression of ER, PR, and HER2 by using standard methods[[20](#_ENREF_20" \o "Chen, 2010 #294)]. Clinico-pathological data were obtained from medical reports of the patients. The current study was approved by the Institutional Review Board (IRB) of the SKMCH&RC (#IRB-16-08) and was exempted from informed consent in agreement with the Declaration of Helsinki Guidelines. We used the specimens of hospital registered patients. The data were recorded in such a manner that the individual identity could not be recognized. This study does not include any procedures that would normally require informed consent outside the context of the study.

***Immunohistochemical staining of FOXP3 and IDO***

Bond III Leica automated system (Leica Biosystems Melbourne, Australia) was used to perform the immunohistochemistry. Briefly, two sections of FFPE blocks of the same patients were obtained. Bond Dewax solution (#AR922, Leica) was used to deparaffinize the slides. Bond ER-2 (#AR9640, Leica) was used to perform heat induced epitope retrieval on the automated system for 20 min. The primary antibodies FOXP3 (Abcam, #ab22510, Cambridge, United Kingdom) or IDO1 (Abcam, #ab55305) were used at a 1:50 and 1:200 dilution, respectively, in primary antibody diluent and incubated for 5 min. BondTM polymer refine detection kit was used to visualize FOXP3 and IDO labeling. Peroxidase block was applied for 5min. The slides were then incubated with post primary rabbit anti mouse immunoglobulin G for 8 min, followed by incubation with polymer anti-rabbit poly-horseradish peroxidase-immunoglobulin G for 8 min. Three prime-diaminobenzidine tetrahydrochloride hydrate was applied for 10 min. Counterstaining was performed with hematoxylin for 5 min. Two pathologists were involved in the study, and they conducted a blind histopathologic assessment. The discrepancies between the two pathologists were reviewed mutually to reach the consensus. The mean score of both pathologists was considered as the final score. Staining of at least 25% of cells was considered positive for FOXP3. FOXP3 expression had nuclear localization[[6](#_ENREF_4" \o "Ladoire, 2011 #462)]. IDO staining evaluation was based on two factors: (1) intensity of cytoplasmic staining (0 to 3); and (2) percentage of cells staining positive (0 to 3). They were categorized as low (1-3), medium (4-6), and high (7-9).

***Statistical analysis***

Statistical analysis was carried out using SPSS software (version 20.0; SPSS, Armonk, NY, United States). For continuous variables, mean and standard deviation were used. For categorical variables, percentages (proportions) were used. Chi-square or Fisher exact test was performed for bivariate analysis. Independent t-test was performed for continuous explanatory variables such as age. Risk factors were identified by using the univariable and multivariable logistic regression model.

**RESULTS**

***Patient baseline characteristics***

A total of 100 breast cancer patients were included in this study with an average age of 48 years. Majority of patients belonged to the Punjab region (88%). Fifty-seven percent of tumors were T2/T3, and 7% tumors were T1 (Tumor Node Metastasis classification). According to the grade distribution, 56% presented grade III. Fifty percent of patients were positive for node, and 49% were positive for metastasis. PR (26%), HER2-neu (26%), and ER (31%) were expressed in the tumor tissue (Table 1). We have further categorized baseline characteristics based on TNBC and hormone positive breast cancer in Table 2.

***Clinicopathological characteristics of breast cancer patients with FOXP3 expression***

There were 25 out of 100 FOXP3 positive cases (Table 3). Based on immunohistochemistry analysis, FOXP3 expression had nuclear localization. All the cases were invasive ductal carcinoma. Furthermore, 18 out of 25 were TNBC patients. The data of 75 out of 100 FOXP3 negative cases are provided in supplementary data (Supplementary Table 1).

***FOXP3 and IDO co-expression is associated with TNBC***

In order to validate the immunosuppressive effect of FOXP3 and IDO co-expression, we categorized the patients into TNBC and hormone-positive breast cancer groups. The mean age at diagnosis of FOXP3 positive *vs* negative breast cancer cases was 47.32 ± 14.19 years and 48.60 ± 11.02 years, respectively (*P* = 0.64). The majority of patients had grade III tumor (*n* = 18) and grade II tumor (*n* = 07). There was a statistically significant association between FOXP3 and high expression of IDO (*P* = 0.01) and TNBC (*P* = 0.01), respectively. Remaining explanatory variables are presented in Table 4.

***FOXP3 and IDO immunostaining***

To evaluate the expression of FOXP3 and IDO, we selected FFPE tumor specimens of the same patients (*n* = 100). Out of 100 patients, 25 expressed FOXP3-positive T-regs, and 75 expressed FOXP3-negative T-regs (Figure 1). IDO positivity was found in all breast tumor specimens. Synchronal expression of FOXP3 and IDO is shown in Figure 1. Immunostaining of low, medium, and high IDO expression is provided in supplementary data (Figure 1).

***Univariable and multivariable analysis***

Table 5 summarizes the several clinicopathological features that were included in unadjusted and adjusted logistic regression model to identify the FOXP3 correlation with IDO expression and TNBC. Two variables were identified as significant independent risk factors for FOXP3 positive: IDO expression high [adjusted odds ratio (AOR) 5.90; 95% confidence interval (CI): 1.22-28.64; *P* = 0.03) and TNBC (AOR 2.80; 95%CI: 0.96-7.95, *P* = 0.05) in multivariable analysis.

**DISCUSSION**

The role of immunosuppression in cancer progression is currently evaluated in various cancers[[21-23](#_ENREF_21" \o "Disis, 2005 #490)]. It has been established that immunological factors such as T-regs are involved in the progression of tumor through induction of immune tolerance in the tumor microenvironment[[7](#_ENREF_7),[22](#_ENREF_22)]. T-regs are effective inhibitors of the immune system[[22](#_ENREF_22" \o "Kalathil, 2016 #503)]. T-regs create immunosuppressive environment by suppressing effector immune cells[[22](#_ENREF_22" \o "Kalathil, 2016 #503)]. They are also associated with poor clinical outcomes in various tumors[[4](#_ENREF_4),[7](#_ENREF_7)]. FOXP3 is a specified marker for T-regs[[2](#_ENREF_2" \o "Curiel, 2008 #460)]. Several studies identified that FOXP3+ T-regs infiltration in tumor microenvironment may affect breast cancer progression[[7](#_ENREF_7),[24](#_ENREF_24)]. Bates *et al*[[7](#_ENREF_7)] demonstrated that a high ratio of FOXP3 cells predict worse relapse-free survival and shorten overall survival in patients with invasive breast carcinoma[[7](#_ENREF_7)]. In another study the researchers observed no difference in overall survival among patients expressing high or low FOXP3[[25](#_ENREF_25)]. There is contradictory data regarding the involvement of FOXP3+ T-regs in breast cancer patients. Nevertheless, we investigated FOXP3 positive *vs* negative expression in the current study. FOXP3 expression was identified in 25 breast cancer patients, and a majority of these patients displayed TNBC phenotype. Overall, 36.73% of TNBC patients expressed FOXP3 positive cells, while 13.72% of hormone positive breast cancer patients expressed FOXP3 positive cells. On the other hand, FOXP3 expression was not detected in 63.26% of TNBC patients and 86.27% of hormone positive breast cancer patients. Our findings of FOXP3 T-regs infiltration in TNBC patients is similar to several studies published before that identified the involvement of FOXP3 positive cells in breast cancer progression[[7](#_ENREF_7),[24](#_ENREF_24)].

FOXP3+ T-regs can restrain effector T cells by an IDO dependent mechanism[[9](#_ENREF_9" \o "Mansfield, 2009 #468)]. IDO plays a critical role in the pathogenesis of breast cancer[[26](#_ENREF_26" \o "Kim, 2017 #299)]. IDO overexpression is linked with shorter overall survival and poor prognosis[[27-34](#_ENREF_27" \o "Brandacher, 2006 #507)]. FOXP3+ T-regs have prognostic implications in TNBC[[8](#_ENREF_8" \o "Lee, 2013 #467)]. IDO expression is also associated with TNBC[[26](#_ENREF_26" \o "Kim, 2017 #299)]. Previously we showed high IDO expression in TNBC patients from Pakistan[[35](#_ENREF_35)]. The aim of our current study was to identify the substantial association between FOXP3-positive T-regs and IDO in TNBC patients. There was a statistically significant association of FOXP3 with high IDO expression (*P* = 0.01) and TNBC (*P* = 0.01) respectively. Two variables were recognized as significant independent risk factors for FOXP3 positive: IDO expression high (AOR 5.90; 95%CI: 1.22-28.64; *P* = 0.03) and TNBC (AOR 2.80; 95%CI: 0.96-7.95; *P* = 0.05) in multivariable analysis. Although several studies focus on the role of immunosuppression in TNBC, our data provide some insight regarding immunosuppression in association with simultaneous expression of FOXP3 and IDO in TNBC patients.

Our study has some limitations, which have to be mentioned. The study population (*n* = 100) did not permit us to draw any strong conclusion. Forthcoming projects on breast cancer patients from Pakistan with inclusive cohort studies are required to authenticate conclusive associations.

Identification of an appropriate immunotherapeutic target for TNBC is currently a hot-topic. FOXP3 and IDO co-expression has the ability to inhibit anti-tumor immune responses and may be considered one of the hurdles in the development of successful immunotherapy for cancer. The role of FOXP3 and IDO co-expression is still a subject of rigorous research in breast cancer.

**CONCLUSION**

In conclusion, the current data revealed that FOXP3 positive cells might be associated with high IDO expression in TNBC patients. FOXP3 and IDO expression monitoring in TNBC patients may provide an effective therapeutic strategy.

**ARTICLE HIGHLIGHTS**

***Research background***

Forkhead box P3 (FOXP3) and indoleamine 2,3-dioxygenase (IDO) are associated with advanced disease in cancer (*e.g.,* breast cancer).

***Research motivation***

To quantify FOXP3 expression in relation with IDO expression in patients diagnosed with breast cancer from Pakistan.

***Research objectives***

Our objective was to identify the co-expression of FOXP3 and IDO in triple negative breast cancer (TNBC) patients.

***Research methods***

Immunohistochemistry was performed to analyze the expression of FOXP3, IDO, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor in human breast cancer tissues.

***Research results***

A significant association of FOXP3 and IDO co-expression was observed among patients with TNBC (*P* = 0.01).

***Research conclusions***

FOXP3 positive cells might be associated with high expression of IDO in TNBC patients.

***Research perspectives***

Evaluation of FOXP3 and IDO expression in TNBC patients may be implemented in the future as a therapeutic strategy.

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**Footnotes**

**Institutional review board statement:** The study was reviewed and approved by the Shaukat Khanum Memorial Cancer Hospital and Research Centre Institutional Review Board.

**Conflict-of-interest statement:** Authors declare no conflict of interests for this manuscript.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at kashifasghar[@skm.org.pk](about:blank). Consent was not obtained but the presented data are anonymized and risk of identification is low. No additional data are available.

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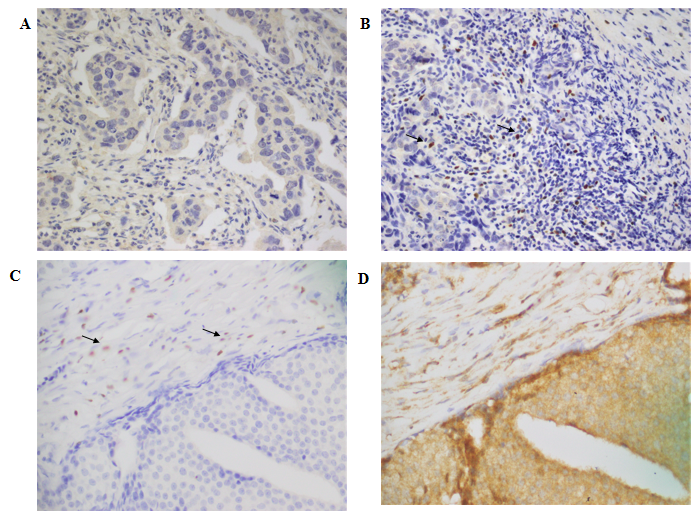
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**Figure Legends**



**Figure 1 Formalin-fixed paraffin-embedded tumor specimens.** A and B: Forkhead box P3 (FOXP3) immunohistochemical staining; A: Invasive ductal carcinoma with FOXP3-negative expression; B: FOXP3-positive lymphocytic infiltration in invasive ductal carcinoma. The staining was nuclear; C and D: Co-expression of FOXP3 and indoleamine 2,3-dioxygenase (IDO), FOXP3 and IDO expression in breast cancer tissues (*n* = 100) were evaluated; C: FOXP3 positive cells infiltrated in invasive ductal carcinoma (nuclear staining). Sections from matched breast cancer patients were stained for IDO; D: Strong and diffuse IDO staining in invasive ductal tumor cells (cytoplasmic staining). Images were captured at × 40 magnification.

**Table 1 Baseline characteristics of breast cancer patients**

|  |  |  |
| --- | --- | --- |
| **Variables** | **Levels** | **Total, *n* %** |
| Age | Mean ± SD | 48.28 ± 11.83 |
| Region | Punjab | 88 (88.0%) |
|  | Khyber Pakhtunkhwa | 7.0 (7.0%) |
|  | Kashmir | 3.0 (3.0%) |
|  | Sindh | 2.0 (2.0%) |
| Histology | Ductal | 91 (91.0%) |
|  | Others | 9.0 (9.0%) |
| Grade | II | 35 (35.0%) |
|  | III | 56 (56.0%) |
|  | UNK | 09 (9.0%) |
| Tumor size | T1 | 7.0 (7.0%) |
|  | T2/T3 | 57 (57.0%) |
|  | UNK | 36 (36.0%) |
| Nodes | Negative | 37 (37.0%) |
|  | Positive | 50 (50.0%) |
|  | UNK | 13 (13.0%) |
| Metastasis | Negative | 38 (38.0%) |
|  | Positive | 49 (49.0%) |
|  | UNK | 13 (13.0%) |
| Estrogen receptor | Negative | 69 (69.0%) |
|  | Positive | 31 (31.0%) |
| Progesterone receptor | Negative | 74 (74.0%) |
|  | Positive | 26 (26.0%) |
| HER2 status | Negative | 74 (74.0%) |
|  | Positive | 26 (26.0%) |
| TNBC | No | 51 (51.0%) |
|  | Yes | 49 (49.0%) |
| Status | Alive | 50 (50.0%) |
|  | Death | 35 (35.0%) |
|  | Lost to follow-up | 15 (15.0%) |
| FOXP3 | Negative | 75 (75.0%) |
|  | Positive | 25 (25.0%) |
| IDO score | Low | 24 (24.0%) |
|  | Medium | 27 (27.0%) |
|  | High | 49 (49.0%) |

UNK: Indicates missing data. FOXP3: Forkhead box P3; HER2: Human epidermal growth factor receptor; IDO: Indoleamine 2,3-dioxygenase; TNBC: Triple negative breast cancer; SD: Standard deviation.

**Table 2 Comparative characteristics of triple negative breast cancer and hormone positive breast cancer patients**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **Levels** | **Triple negative breast cancer, *n* (%)** | **Hormone positive breast cancer, *n* (%)** |
| Age | Mean ± SD | 47.24 ± 11.5 | 49.27 ± 12.0 |
| Histology | Ductal | 46 (50.5) | 45 (49.5) |
|  | Others | 3 (33.3) | 6 (66.7) |
|  | Total | 49 (49.0) | 51 (51.0) |
| Grade | II | 9 (25.7) | 26 (74.3) |
|  | III | 37 (66.1) | 19 (33.9) |
|  | UNK | 3 (33.3) | 6 (66.7) |
|  | Total | 49 (49.0) | 51 (51.0) |
| Tumor size | T1 | 4 (57.1) | 3 (42.9) |
|  | T2/T3 | 26 (45.6) | 31 (54.4) |
|  | UNK | 19 (52.8) | 17 (47.2) |
|  | Total | 49 (49.0) | 51 (51.0) |
| Nodes | Negative | 23 (62.1) | 14 (37.8) |
|  | Positive | 21 (42.0) | 29 (58.0) |
|  | UNK | 5 (38.4) | 8 (61.6) |
|  | Total | 49 (49.0) | 51 (51.0) |
| Metastasis | Negative | 23 (60.5) | 15 (39.5) |
|  | Positive | 21 (42.8) | 28 (57.2) |
|  | UNK | 5 (38.4) | 8 (61.6) |
|  | Total | 49 (49.0) | 51 (51.0) |

UNK: Indicates missing data. SD: Standard deviation.

**Table 3 Clinicopathological characteristics of breast cancer patients with nuclear forkhead box P3 expression**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Case** | **Histology** | **Age in yr** | **Grade** | **Nodes** | **Metastasis** | **ER** | **PR** | **HER2** | **TNBC** |
| 1 | Ductal | 28 | 3 | 0 | **-** | **-** | **-** | **-** | **+** |
| 2 | Ductal | 54 | 3 | 14 | **+** | **-** | **-** | **-** | **+** |
| 3 | Ductal | 67 | 3 | 1 | **+** | **-** | **-** | **-** | **+** |
| 4 | Ductal | 65 | 2 | UNK | UNK | **-** | **-** | **-** | **+** |
| 5 | Ductal | 45 | 3 | 13 | **+** | **-** | **-** | **-** | **+** |
| 6 | Ductal | 45 | 3 | 0 | **-** | **-** | **-** | **-** | **+** |
| 7 | Ductal | 55 | 3 | 0 | **-** | **-** | **-** | **-** | **+** |
| 8 | Ductal | 23 | 3 | 0 | **-** | **-** | **-** | **-** | **+** |
| 9 | Ductal | 47 | 2 | 0 | **-** | **-** | **-** | **-** | **+** |
| 10 | Ductal | 73 | 3 | 2 | **+** | **-** | **-** | **-** | **+** |
| 11 | Ductal | 35 | 3 | 0 | **-** | **-** | **-** | **-** | **+** |
| 12 | Ductal | 35 | 3 | 0 | **-** | **-** | **-** | **-** | **+** |
| 13 | Ductal | 52 | 3 | UNK | UNK | **-** | **-** | **-** | **+** |
| 14 | Ductal | 39 | 3 | 0 | **-** | **-** | **-** | **-** | **+** |
| 15 | Ductal | 48 | 3 | 0 | **-** | **-** | **-** | **-** | **+** |
| 16 | Ductal | 70 | 3 | 2 | **+** | **-** | **-** | **-** | **+** |
| 17 | Ductal | 40 | 3 | 13 | **+** | **-** | **-** | **-** | **+** |
| 18 | Ductal | 35 | 3 | 13 | **+** | **-** | **-** | **-** | **+** |
| 19 | Ductal | 43 | 3 | 0 | UNK | **-** | **-** | **+** | **-** |
| 20 | Ductal | 36 | 2 | 1 | **+** | **-** | **-** | **+** | **-** |
| 21 | Ductal | 45 | 3 | UNK | UNK | **+** | **+** | **-** | **-** |
| 22 | Ductal | 71 | 2 | 6 | **+** | **+** | **-** | **-** | **-** |
| 23 | Ductal | 30 | 2 | 17 | **+** | **+** | **+** | **-** | **-** |
| 24 | Ductal | 40 | 2 | UNK | UNK | **-** | **-** | **+** | **-** |
| 25 | Ductal | 62 | 2 | 0 | **-** | **+** | **+** | **+** | **-** |

UNK: Indicates missing data; Grade: Nottingham Histologic Score; Nodes: No. of nodes involved. ER: Estrogen receptor; HER2: Human epidermal growth factor receptor; PR: Progesterone receptor; TNBC (+): Triple negative breast cancer; TNBC (**-**): Hormone-positive breast cancer.

**Table 4 Patients and tumor characteristics of forkhead box P3 negative *vs* positive**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variables** | **Characteristics** | **FOXP3 Negative 75 (75.0%)** | **FOXP3 Positive**  **25 (25.0%)** | ***P* value** |
| Age (yr) | Mean ± SD | 48.60 ± 11.02 | 47.32 ± 14.20 | 0.64 |
| IDO score | Low | 22 (91.7%) | 2 (8.3%) | 0.01a |
|  | Medium | 23 (85.2%) | 4 (14.8%) |  |
|  | High | 30 (61.2%) | 19 (38.8%) |  |
| Grade | II | 28 (80.0%) | 7 (20.0%) | 0.21 |
|  | III | 38 (67.9%) | 18 (32.1%) |  |
| Metastasis | Negative | 27 (71.1%) | 11 (28.9%) | 0.45 |
|  | Positive | 39 (79.6%) | 10 (20.4%) |  |
| Tumor size | T1 | 4 (57.1%) | 3 (42.9%) | 0.15 |
|  | T2/T3 | 46 (80.7%) | 11 (19.3%) |  |
| Lymph nodes involvement | Negative | 26 (70.3%) | 11 (29.7%) | 0.29 |
|  | Positive | 40 (80.0%) | 10 (20.0%) |  |
| Estrogen receptor | Negative | 48 (69.6%) | 21 (30.4%) | 0.06 |
|  | Positive | 27 (87.1%) | 4 (12.9%) |  |
| Progesterone receptor | Negative | 52 (70.3%) | 22 (29.7%) | 0.06 |
|  | Positive | 23 (88.5%) | 3 (11.5%) |  |
| HER2–neu receptor | Negative | 53 (71.6%) | 21 (28.4%) | 0.19 |
|  | Positive | 22 (84.6%) | 4 (15.4%) |  |
| Triple negative breast cancer | No | 44 (86.3%) | 7 (13.7%) | 0.01a |
|  | Yes | 31 (63.3%) | 18 (36.7%) |  |

a*P* < 0.05. IDO: Indoleamine 2,3-dioxygenase; HER2: Human epidermal growth factor receptor; FOXP3: Forkhead box P3; SD: Standard deviation.

**Table 5 Univariable and multivariable logistic regression analysis for forkhead box P3-negative (reference) *vs* forkhead box P3-positive**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **Characteristics** | **Univariable analysis odds ratio (95% CI), *P* value** | **Multivariable analysis odds ratio**  **(95% CI), *P* value** |
| IDO score | Low | Ref. | Ref. |
|  | Medium | 1.91 (0.32-11.52), 0.50 | 2.32 (0.37-14.50), 0.37 |
|  | High | 6.97 (1.50-33.10), 0.01a | 5.90 (1.22-28.64), 0.03a |
| Triple negative breast cancer | No | Ref. | Ref. |
|  | Yes | 3.65 (1.36-9.80), 0.01a | 2.80 (0.96-7.95), 0.05a |

a*P* < 0.05. IDO: Indoleamine 2,3-dioxygenase.