Status of indoleamine-2,3-dioxygenase 1 in infiltrating ductal carcinoma of breast cancer: a new prognostic indicator for aggressive tumors

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STATUS OF INDOLEAMINE-2,3-DIOXYGENASE 1 IN INFILTRATING DUCTAL CARCINOMA OF BREAST CANCER: A NEW PROGNOSTIC INDICATOR FOR AGGRESSIVE TUMORS

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Abstract – *Objective:* *The tryptophan pathway has been demonstrated to be involved in tumor progression. Increased expression of indoleamine 2,3-dioxygenases has been observed in several human tumors types, such as breast cancer. In order to study the role of IDO expression as a prognostic marker, the serum tryptophan and kynurenine concentrations and the ratio of kynurenine to tryptophan (K/T) of 165 subjects were compared, and correlations were established.*

Patients and Methods: *This is a case-control study involving 39 patients with invasive ductal breast carcinoma. The recruitment period was from January 2018 to December 2019. We investigated the serum tryptophan and kynurenine levels based on liquid chromatography-tandem mass spectrometry (LC-MS/MS).*

Results: *Our analysis shows a faster tryptophan degradation in larger tumors. The K/T ratio was significantly associated with breast cancer risk factors (age, tumor size and biomarkers). It was also significantly associated with KI67 (r=0.208;* p=*0.007) and with age (r= 0,166* p=*0,033) as well. The plasmatic K/T ratio expression greater than 8.4 µmol/ µmol was significantly associated with an age of 63±14 years old (OR=1.05, 95% IC 1.01-1.09,* p=*0.02). Regression analysis also shows that higher plasmatic K/T ratio expression was associated two times with higher tumour size and SBR grade (OR=2.11, 95% IC 0.95-2.83,* p=*0.035; OR=2, 95% IC 1.11-3.54,* p=*0.02, respectively). Subjects with a high K/T ratio (>6.2) are associated five times with RE+ and RP+ compared to subjects with a low K/T ratio (<4.2) (OR=5, 95% IC 1.23-18.81,* p=*0.02; OR=4.2, 95% IC 1.07-16.72,* p=*0.04, respectively). It was also significantly associated with higher KI67 expression (ki67≥20) (OR=1.14, 95% IC 1.04-1.26,* p=*0.008).*

Conclusions: *The K/T ratio can be used as a new prognostic biomarker for the progression and invasiveness of breast invasive ductal carcinoma.*

KEYWORDS: *Kynurenine, Tryptophan, Biomarkers, Infiltrating ductal carcinoma.*

LIST OF ABBREVIATIONS: *IDO- Indoleamine 2,3-Dioxygenase, TRP- tryptophan, KYN- kynurenine, K/T- kynurenine-to-tryptophan ratio, LC-MS/MS- Liquid Chromatography coupled with Tandem Mass Spectrometry, BC- Breast cancer, HR- Hormone Receptors, ER - Estrogen receptor, PR - Progesterone receptor, CTRL- Healthy Controls, OC- oral contraceptives, T1-tumour size ≤2 cm, T2-tumour size between 2 and 5 cm, T3- size >5 cm, N0- node-negative, N1- node-positive, SBR- Scarff Bloom and Richardson, r-Correlation coefficient, OR: odds ratio, CI: confidence interval, Q: quartile, IDC-infiltrating ductal carcinoma.*

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INTRODUCTION

Breast cancer (BC) is a remarkably complex biologically and clinically heterogeneous disease. This heterogeneity can be observed at every inspection level from macroscopic to molecular. BC is initially a localized disease, but after different types of treatments (mastectomy, radiotherapy, and chemotherapy), it can metastasize to lymph nodes and distant organs[1-3]. During tumor progression and metastasis, both tumor and stromal cells undergo rapid metabolic adaptation[2]. Over evolution, some amino-acid metabolism, such as arginine and tryptophan (TRP) in different cells have become critical checkpoints therapy efficacy in patients with cancer[4,5]. In mammalian cells, the pathway kynurenine (KYN) is initiated by the rate-limiting enzyme tryptophan-2,3-dioxygenase (TDO or TDO2) and interferon responsive indoleamine 2,3-dioxygenase (IDO) and is the major route for TRP catabolism[6]. IDO1 regulates immune cell function through the kyn pathway, but it consumes TRP in the microenvironment, especially in tumors, which has led to the development of IDO1 inhibitors for cancer therapy[6]. The prognosis of BC depends on several clinical and pathological parameters. The most important is clinical stage. Other predictive factors are hormone receptor (HR), tumor size (T), HER2 and KI67[7]. Changes in blood TRP and KYN levels have been examined in a variety of human cancers[8,9]. The immunosuppressive enzyme IDO is overexpressed in many different tumor types including BC[10]. Cancer research provides some support for the role of increased TRP degradation in systemic immune activation, the physical symptoms severity, and the immune cells regulation in the tumor microenvironment and in the disease progression[11-13]. Altered TRP metabolism, also associated with poor disease outcomes and adverse neuropsychiatric symptoms, has received little interest in women with BC; however, women with BC have neuropsychiatric symptoms increased rates, not only during the acute treatment phase but for some into survivorship[14,15]. A retrospective analysis conducted on 203 BC cases showed that none of the tumors were negative for IDO staining in immunohistochemistry[9]. Moreover, node-positive (N+) and estrogen receptor-positive (ER+) tumors were associated with higher IDO expression, while greater vascularised tumors showed lower IDO staining[9]. Another study reported a negative association between high stromal IDO and worse disease-free and metastasis-free survival in BC[16]. Wei et al[11] measured IDO mRNA expression for BC cell lines in the MCF-7 supernatants. They found that IDO facilitated angiogenesis in BC[16]. Patterson et al (17) found that ERɤ negatively regulated IDO expression. After analyzing 14 BC data sets, Dewi et al[18] found that in ER+ tumors, the expression of IDO is lower than that of ER- cells, and they speculated that ER may inhibit IDO by promoting IDO1 methylation[17,18]. Kim et al[19] found that IDO positivity was unevenly distributed among Triple-negative BC subtypes and that IDO positive tumors had more active TIL responses than did IDO negative tumors[19]. Another more recent study showed lower plasmatic TRP and KYN levels in BC cases than in healthy controls (CTRL), mainly in ER- tumors and at a large tumor stage[20]. In addition, patients with advanced lung cancer had significantly higher IDO activity and lower TRP concentrations than those in the early stages, suggesting that TRP degradation may occur more significantly with advanced stage cancer[13]. Tang et al[21] observed higher KYN plasmatic levels in ER− compared to ER+ BC. Overall, there has been little research on TRP degradation in BC patients. The aforementioned studies did not establish the correlation between tumor characteristics and plasma KYN, TRP and K/T ratios, compared with those found in other diseases[22,23]. As far as we know, two studies in BC have determined the correlation between plasma KYN, TRP, K/T ratio and tumor characteristics (SBR grade, TNM, HR, HER2, KI67)[9-24]. The purpose of our study is to first analyze the TRP and KYN level in the plasma of IDC and CTRL patients, and then test the association of IDO levels with tumor aggressiveness and BC biomarkers (HR, HER2, KI67).

PATIENTS AND METHODS

This is a case-control study of 39 IDC patients and 126 CTRL patients recruited in Tlemcen Gynecology. The data are collected at the time of diagnosis. The information collected includes sociodemographic, clinical, histological data (TNM classification, SBR grade) and BC biomarkers (HR, HER2, KI67). To test tumor aggressivety we used tumor size, grade and KI67. The histological grade was assessed using the Nottingham grading system[25]. Tumor staging was based on the 7th American Joint Committee on Cancer criteria[26]. A blood sample was collected for the evaluation of serum TRP and KYN levels. The K/T ratio was used to express the IDO activity level. Subjects are only eligible to participate in the study after obtaining consent. This study protocol was approved by the Ethics Committee of the University of Tlemcen (Algeria).

DETERMINATION OF SERUM TRYPTOPHAN AND KYNURENINE LEVELS

Serum TRP and KYN concentrations were measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using already described method (27,28).

50 µL of serum were mixed and centrifuged (11800 rpm, at +4°C, for 8 min), after the addition of 50 µL of acetonitrile for protein precipitation, containing TRP D5 at 50 µM (CDN isotopes, Pointe-Claire, Canada), as an internal standard. 50 µL of supernatant is taken up and added vial wise to deionized water (60 µL). 10 µL of this mixture is finally injected into a UPLC®-MS/MS system (Acquity TQ-XS Detector, Waters, Milford, CT, USA) equipped with a 2.1x100 mm-1.7 µm CSH Phenyl-Hexyl column (Waters, Milford, CT, USA). The ESI+ (Positive Electrospray Ionization) and MRM (Multiple Reactions Monitoring) modes are used respectively for ionization and detection of each transition of the molecules to be quantified. The MassLinks (Waters) software is used for data acquisition and processing.

The serum K/T ration level was divided into quartiles (Q1=4.9, median=6.24, Q3=8.32, maximum=42.88).

STATISTICAL ANALYSIS

Statistical analyses were performed using the Minitab Version 16 statistical software package. Descriptive statistics for each variable were performed. Clinical, histopronostic and BC biomarkers characteristics were compared as a function of serum TRP, KYN, and K/T ration. The data distribution normality was assessed using the Kolmogorov-Smirnov test. Statistically significant difference between groups was determined by parametric statistics (*t*-test) test for categorical variables. We performed correlations between serum TRP, KYN and K/T ration levels, according to continuous parameters (age, RE,RP, ki67). The logistic regression model was used to assess the univariate association between plasmatic K/T ration expression and the various clinical, pathologic and biomarkers factors that were included in the univariate statistics. A value of $p< 0.05$ was considered statistically significant.

RESULTS

There are a total of 165 subjects, of which 126 are CTRL, 39 of them are IDC Phase II-III, and all participants are women. Among 39 breast cancer patients, 7 (18%) had a family history of BC. We did not observe any differences in circulating KYN, TRP and the K/T ratio distribution between BC patients than CTRL, as shown in Table 1. The mean ages was 54±10 and 59±12 years old for 39 BC patients and the CTRL group respectively.

The patients were under the age of 45 years old in 15% of the cases. According to the TNM classification, the patients were classified T1 in 5%, T2 in 74%, T3 in 13% and T4 in 5% of the cases. The patients were classified as stage II in 82% and stage III in 18% of the cases. For HR, ER+ was expressed in 79%, PR in 74%, HER2+ in 13% and Ki67≥20% in 33% of cases.

TRP value in plasma was 54.45±18.96 µmol/L and 60.82±24.01 µmol/L in the BC and CTRL, respectively. KYN value in plasma, was 4±1.6 µmol/L and 4.01±1.9 µmol/L in the BC and CTRL, respectively, and for the plasmatic (K/T)*100 ratio was 8±4.2 (µmol/ µmol) for the BC patients and 7±4.8 (µmol/µmol) for the CTRL. The comparison between the two patient groups for clinical, histopronostic and biomarkers data is presented in Table 1.

We observed a progressively lower circulating TRP level according to the tumor size increase from 69.564±20.41 µmol/L in T1 to 53.639±19.134 µmol/L in (T2-T4) (p=0.00). It was also significant lower TRP for the subject with ki67≥20 compared to subject with ki67 [10-19] (p=0.005) (Table1). However, no significant association was seen for the other parameters (Table 1). The KYN in plasma showed a different statistical significant according to the age of menopause. Circulating KYN was significantly higher in the premenopausal patients than in the postmenopausal patients with a mean value of 4.29±1.68 µmol/L; 3.25±1.10 µmol/L (p=0.03), respectively (Table1).

The K/T ratio in plasma showed a different statistically significant according to most of clinicopathological parameters and all BC biomarkers (p<0.05) (Table 1).

We have noticed a higher plasmatic K/T ratio in older patients (≥45 years old) (p=0.001). We have also observed a statistically significant higher circulating K/T ratio according to larger tumors. In stage T1 tumors (5.5±0.79 µmol/µmol) compared to T2-T4 tumors (8.1±4.3µmol/µmol) have a higher plasmatic K/T ratio level (p=0.029 µmol/µmol) (Table 1). A statistically significant higher K/T ratio in plasma was observed for patients with node-positive compared to patients with node-negative. There was no statistically significant relationship between the K/T ratio level and SBR grade (p>0.05) (Table 1).

TABLE 1. KYN, TRP and K/T ratio according to tumor characteristics.

Variables Mean ± SD	*Serum TRP (µmol/L)*	*Serum KYN (µmol/L)*	*(K/T)*100 (µmol/ µmol)*
Healthy vs. cancer			
Healthy	60.82±24.01	4.01±1.99	7.36±4.82
Cancer	54.46±18.96	4.00±1.60	7.95±4.21
p-value	0.130	0.97	0.49
Age group (years)			
≤45 years	46.61±17.19	2.96±1.28	6.5±1.49
>45 years	57.79±19.54	4.30±1.55	8.1±3.92
p-value	0.198	0.05	**0.001**
Age of menopause			
<50 years	49.26±13.78	3.25±11.03	6.73±1.67
≥50 years	56.49±20.50	4.29±16.86	8.44±4.81
p-value	0.210	**0.03**	0.109
OC			
Presence	45.84±18.28	3.60±1.48	8.61±3.99
Absence	58.28±18.29	4.18±1.64	7.66±4.80
p-value	0.050	0.300	0.520
Tumor size Grade			
T1	69.56±20.41	3.84±6.64	5.5±0.79
[T2-T3-T4]	53.63±19.13	4.01±1.64	8.1±4.28
p-value	**0.000**	0.810	**0.029**
N stage			
N0	60.49±24.72	3.45±1.57	5.7±0.62
N1	53.76±18.52	4.06±1.61	8.2±4.37
p-value	0.500	0.470	**0.000**
Tumor SBR stage			
II	55.22±55.22	3.92±1.52	7.7±4.16
III	50.93±50.93	4.34±2.03	9.3±4.51
p-value	0.590	0.530	0.373
RE			
Positive	53.15±17.42	3.97±15.70	8.2±4.62
Negative	59.49±24.80	4.11±18.29	7.1±1.96
p-value	0.490	0.850	**0.002**
RP			
Positive	53.47±17.625	4.0±1.59	8.2±4.69
Negative	57.72±23.803	4.01±1.73	7.1±1.84
p-value	0.560	0.980	**0.001**
HER-2-neu			
Positive	56.85±22.48	4.10±2.02	7.1±1.15
Negative	54.10±18.75	3.98±1.56	8.1±4.49
p-value	0.76	0.880	**0.000**
KI67			
[10-20[	60.35±16.90	3.74±1.12	6.3±1.38
≥20	42.66±17.81	4.52±2.25	11.3±5.8
p-value	**0.000**	0.150	**0.000**

Key: **OC**: oral contraceptives, **T1**: tumour size ≤2 cm, **T2**: tumour size between 2 and 5 cm, **T3**: size >5 cm. **N0**: node-negative, **N1**: node-positive, **SBR**: Scarff Bloom and Richardson, **RE**: estrogen receptor, **RP**: progesterone receptor.

In the cohort of cancerous patients, a statistically significant higher K/T ratio in plasma was observed for the HR+ and HER2- patients compared to HR- and HER2+ patients, respectively (Table 1).

Concerning the KI67 proliferation index, a statistically significant higher K/T ratio in plasma was seen for patients with KI67≥20 compared to patients with KI67 ((11.3±5.88; 6.3±1.38 µmol/µmol), *p*=0.00, respectively) (Table 1)

TRP value in plasma was negatively associated with KI67 (r=-0.187; *p*=0.018) (Table 2). However, there was no significant association between age and RE (*p*>0.05) (Table 2). The KYN was positively associated with KI67 (r=0.320; *p*=0.000) (Table 2). The K/T ratio was also significantly associated with age (r= 0.166; *p*=0.033) and with KI67 (r=0.208; *p*=0.007) (Table 2).

Table 3 summarizes the various clinical, pathologic and biomarkers factors that were included in

TABLE 2. Correlation of KYN, TRP and K/T ratio with the age, RE, RP and Ki67.

Variables	*TRP*		*KYN*		*K/T ratio*	
	r	*p-value*	*r*	*p-value*	*r*	*p-value*
Age	0.092	0.239	0.320	**0.00**	0.166	**0.03**
RE	-0.129	0.098	-0.000	0.90	0.069	0.37
RP	-0.103	0.187	-0.001	0.99	0.058	0.45
KI67	-0.184	**0.018**	0.083	0.28	0.208	**0.00**

Key: **r**: Correlation coefficient, **RE**: estrogen receptor, **RP**: progesterone receptor.

the univariate statistical analysis to explore their relationship with K/T ratio expression. However, univariate analysis for higher K/T ration expression between age, tumor size, node lymph, stage SBR, RH and KI67 was associated compared to lower K/T ratio expression. There was no significant association between higher K/T expression and other factors (Table 3).

The logistic model significantly retained the age of diagnostic (OR=1.05, 95% IC 1.01-1.09, *p*=0.02). A higher plasmatic K/T ratio expression than 8.4 µmol/ µmol is associated with age of 63±14 years old compared to patients with lower plasmatic K/T ratio expression. Concerning tumor aggressiveness, a higher plasmatic K/T ratio expression is associated two times with larger tumor size and grade III than subjects with K/T lower (<4,9) (OR=2.11, 95% IC 0.95-2.83, *p*=0.035; OR=2, 95% IC 1.11-3.54, *p*=0.02, respectively). K/T ratio expression greater than 8.4 (µmol/ µmol) is associated four times more for patients with node-positive (OR=4, 95% IC 1.21-11.65, *p*=0.02) compared to patients with low K/T ratio. The logistic model also significantly retained the BC biomarkers subjects with a K/T ratio higher (>6.2) are associated five times more exposed RE+ and RP+ compared to subjects with a low K/T ratio (<4.2) (OR=5, 95% IC 1.23-18.81, *p*=0.02; OR=4.2, 95% IC 1.07-16.72, *p*=0.04, respectively). In addition, subjects with a K/T ratio higher (K/T ratio>4.9) were associated with higher KI67 expression (ki67≥20) compared to subjects with lower K/T ratio expression (K/T ratio<4, 9) (OR=1.14, 95% IC 1.04-1.26; *p*=0.008). No relationship was seen between K/T ration expression and clinical factors such as, age, menopausal status and HER2 (*p*>0,05).

TABLE 3. The different quartiles of K/T ratio expression according to tumor characteristics.

	Coeff	*Coeff ErT*	*Z*	*p*	*OR*	*CI 95%*
Age: (Q4/Q1)	0.046	0.020	2.29	**0.022**	1.05	1.01-1.09
Age: (Q3/Q1)	-0.001	0.019	-0.06	0.951	1.00	1.00-1.04
Age: (Q2/Q1)	-0.003	0.018	-0.21	0.836	1.00	0.96-1.03
T: (Q4/Q1)	0.456	0.259	1.76	0.078	1.58	0.95-2.62
T: (Q3/Q1)	0.539	0.255	2.11	**0.035**	2.11	0.95-2.83
T: (Q2/Q1)	0.451	0.257	1.75	0.08	1.57	0.95-2.60
N: (Q4/Q1)	1.322	0.578	2.29	**0.021**	3.75	1.21-11.65
N: (Q3/Q1)	1.128	0.587	1.92	0.055	3.09	0.98-9.78
N: (Q2/Q1)	0.771	0.60	1.27	0.204	2.16	0.66-7.10
SBR:(Q4/Q1)	0.597	0.298	2.00	**0.046**	1.82	1.01-3.26
SBR:(Q3/Q1)	0.684	0.296	2.31	**0.021**	1.98	1.11-3.54
SBR:(Q2/Q1)	0.617	0.296	2.08	**0.037**	1.85	1.04-3.31
RE: (Q4/Q1)	1.121	0.717	1.56	0.118	3.07	0.75-12.53
RE: (Q3/Q1)	1.569	0.696	2.25	**0.024**	4.80	1.23-18.81
RE: (Q2/Q1)	1.209	0.707	1.71	0.087	3.35	0.84-13.41
RP: (Q4/Q1)	0.958	0.729	1.31	0.189	2.61	0.62-10.89
RP: (Q3/Q1)	1.440	0.702	2.05	**0.040**	4.22	1.07-16.72
RP: (Q2/Q1)	1.209	0.707	1.71	0.087	3.35	0.84-13.41
KI67:(Q4/Q1)	0.131	0.049	2.66	**0.008**	1.14	1.04-1.26
KI67:(Q3/Q1)	0.115	0.049	2.31	**0.021**	1.12	1.02-1.24
KI67:(Q2/Q1)	0.106	0.049	2.14	**0.032**	1.11	1.01-1.23

Key: **OR**: odds ratio, **CI**: confidence interval, **Q**: quartile, **T1**: tumour size ≤ 2 cm, **T2**: tumour size between 2 and 5 cm,**T3**: size >5 cm, **N0**: node-negative, **N1**: node-positive, **SBR**: Scarff Bloom and Richardson, **RE**: estrogen receptor, **RP**: progesterone receptor.

DISCUSSION

This study analyzed the KYN and TRP levels and their ratios in IDC and CTRL patients. Based on the characteristics observed in Algeria and Africa, this is the first study to determine the difference in circulating TRP and its metabolite KYN levels in the presence of BC. IDC's plasma.

K/T ratio expression comparison in different histological breast cancer types was significantly different with a *p*-value of 0.01[24]. K/T ratio is higher than that of lobular carcinoma and other histology[24].

A recent research that analyzed 220 BC cases and 146 CTRL IDO expression published a study by Onesti et al[24]. They observed that the TRP, KYN and IDO levels are significantly lower in plasmatic BC patients compared to CTRL[24]. These results are not consistent with previously published study by Lyon et al[8] that shows no different statistically significant plasmatic TRP, KYN levels in BC patients compared to CTRL[8]. This result is consistent with our findings.

Since 1967, age and sex in BC have influenced the metabolism of TRP[29]. This study shows that TRP metabolism was higher in young cases between the ages of 21years and 36 years compared to men and women over 40 years old[29]. Another study also shows a higher KYN plasmatic concentration and K/T ratio for patients older than 54 years old[24]. In our study, regression analysis shows that the K/T ratio expression is associated with the age of 63±14 years. This result is consistent with literature data showing an increase in TRP metabolism with age[24,30].

The BC staging at diagnosis is used to check whether plasma TRP or KYN is related to BC aggressiveness[20]. Both TRP and KYN were significantly lower in grade III/IV BC patient plasma than in plasma from CTRL, while there was no difference in either TRP or KYN when comparing grade 0/I patients *vs.* CTRL[20]. This is consistent with the results of Onesti et al[24], who observed that TRP degradation in larger tumors was faster, compared with grade II and III tumors, and grade I tumors and T1 tumors were compared with T2-4 tumors. They observed a higher plasmatic TRP level[24]. This is consistent with our finding showing a lower circulating TRP level in T3-T4. Another study showed that the expression of kynurinase (an enzyme that degrades KYN) was inversely related to grading tumoral[31]. This study concluded that kynurinase suppresses BC cell proliferation, growth and tumor development. Kynurinase may function as a tumor suppressor in BC[31]. In the study by Soliman et al[9], the Univariate logistic regression for IDO scores used revealed that larger tumors (OR = 0.45, 0.22–0.92, *p*= 0.028) and node-positive tumors. (OR = 0.51, 0.28–0.93, *p* = 0.028) were associated with lower IDO expression[9]. This is inconsistent with the lower cyclic K/T ratio level we found. It has been shown a strong difference in relation to the HR status of 18% metabolites[21]. The precursor molecule TRP did not vary between ER + and ER- cancers, whereas median levels of KYN were significantly elevated in ER- tumors[21]. Another interesting finding is that in HR-tumors, lower circulating TRP levels are accompanied by higher K/T ratio[24]. In contrast, the study by Green et al[20] shows that TRP, KYN and IDO were lower in the plasma from BC patients with ER-[20]. In addition, although serum KYN levels and tumor IDO1 expression in ER+ patients are lower than ER-tumors, IDO1 is negatively correlated with ER[17]. Our study shows that the K/T ratio level is higher in the plasma from BC patients with ER+ patients than ER- tumors and that there is no relationship between TRP, KYN levels and HR. The same results were shown by Soliman et al[9] in a cohort of 203BC patients' cases[9]. However, this study was carried out in greater Baltimore area on a population in which 61.1% of cases were of African America, ER+ tumors were associated with higher IDO expression than ER- tumors (OR = 2.25, 1.26–4.04, *p* = 0.006). Regarding HER2, published studies are more interested in RH than HER2 because their goal is to treat[17,32]. In the literature, there are no differences between KYN, TRP and the K/T ratio levels and HER2[9,24]. Univariate analysis for high IDO expression between BC types demonstrated triple-negative tumors (OR = 0.29 (0.08–1.08, *p* = 0.06) and HER2+ (OR = 0.76 (0.33–1.72, *p* = 0.51) compared to ER+/HER2- tumors (9). In addition, Univariate analysis for lower IDO and HER2 (OR= 1.09, IC (0.52-2.29, *p* = 0.8262)[9].

The rate of proliferative mitotic index KI67 is a key phenotypic characteristic of BC and can be used as an independent prognostic factor[33]. The rate of proliferation affects the levels of many metabolites[33]. A correlation has been shown of ki67 with 399 different metabolites levels[21]. The results showed that there is a strong correlation between the number of metabolites found in fast-growing cells and the rate of KI67[21]. In our study we found a positive correlation between K/T ration level and KI67 (r=0.208; *p*=0.007) and negative correlation between TRP level in plasma and KI67(r=-0.187; *p*=0.018)[21]. Our results also show that patients with high KI67 ≥20 have a higher K/T ration (11.3±5.88 µmol/ µmol) compared to patients with lower KI67 [10-20] (6.3±1.384 µmol/µmol) and increased TRP degradation in patients with a higher KI67 (≥20).

These results are not consistent with the results of Onesti et al[24]. They observed no difference in KI67 expression in the cycle of KYN, TRP, and K/T ratio[24]. Supervised clustering and correlation analysis of the metabolites with proliferation rate demonstrated sets of metabolites that were positively and inversely correlated with proliferation[21].

CONCLUSIONS

Our findings confirm that the reduction of serum tryptophan concentration plays a vital role in tumor progression. In addition, our data shows that aggressive tumors have high levels of tryptophan degradation. A higher K/T ratio is associated with aggressive tumors and biomarkers (HR, HER2, KI67). The elevation of breast cancer risk was especially in women with invasive ductal carcinoma with estrogen receptor positive (RE+), progesterone receptor positive (RP+) and high KI67 expression (ki67≥20). According to the above observations, tryptophan metabolism is involved in the heterogeneity of breast cancer.

Acknowledgemen:
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Ethical approval:
The study was approved by the Ethics Committee of the university of Tlemcen, Algeria.

Conflict of interest:
Authors declare that they have no conflict of interest.

Informed consent and patient details:
The authors declare that this report does not contain any personal information that could lead to the identification of the patient(s) and/or volunteers.

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