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RESEARCH ARTICLE

High expression of the checkpoint molecule PD-1 on regulatory and helper CD4$^+$ T cells in metastatic breast cancer patients with poor prognosis

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ABSTRACT

Background: Recurrence and metastasis are common in patients of breast cancer even after chemotherapy and radiotherapy. This recurrence may occur due to the emergence of immunoregulatory T (T_{reg}) cells and the immune checkpoint molecules such as programmed cell death protein 1 (PD-1) which are known to suppress anti-tumor immunity. Aim: The aim of this study is to investigate the numbers of T_{reg} cells and the expression of PD-1 molecule on T cells in the peripheral blood of breast cancer patients at different stages (II, III, and IV) compared to healthy individuals. **Materials and Methods**: Blood samples were collected from early diagnosed metastatic breast cancer patients before surgery. Leucocytes were stained with different antibodies including CD4, CD25, CD127, and PD1, and then were analyzed by multiparametric flow cytometry. **Results:** The results showed that the relative and absolute numbers of T_{reg} cells were significantly increased in breast cancer patients. Interestingly, we found a correlation between the numbers and cancer progression from stages II to III and IV, as compared with healthy individuals. Furthermore, significant increases in PD-1 expression were observed on T_{reg} cells and CD4$^+$ T cells in cancer patients as compared to healthy controls. This profile of PD-1 expression was also correlated with the poor prognosis of the patients. **Conclusion**: The results provide a better understanding of the immunomodulatory role of T_{reg} cells and PD-1 expression in breast cancer patients who are the target for immunotherapy using checkpoint inhibitors.

Keywords: Breast cancer, T lymphocyte, T regulatory cells, Checkpoint molecules, Programmed cell death protein, PD-1, Flow cytometry

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INTRODUCTION

Breast cancer is the most common cancer among women, approximately 2.1 million women each year. Furthermore, 627,000 women died from this cancer, resemble 15% of all cancer deaths among women (WHO, 2019). Recurrence and metastasis are common in patients with breast cancer and still obscure in treatment. One possible mechanism suggests suppression of the immune cells by several immunoregulatory (checkpoints) molecules, which expressed on immune cells as well as cancer cells (Chawla et al., 2014). The immune system has evolved an array of regulatory mechanisms to protect against tissue damage from autoimmunity or during the active response to the pathogen. These protective mechanisms are mediated by inhibitory receptors or called immune checkpoints Like CTLA-4 and PD-1, which induced upon effector

T cell activation. The checkpoints are also highly expressed on regulatory T cells (T_{reg}) provided a negative feedback mechanism that is crucial for immunoregulation and protection of tissues from an over-exuberant inflammatory response (Leone et al., 2015). Regulatory T cells with phenotypes ($CD4^+CD25^+$) are a component of the immune system that involved shutting down immune responses after they have successfully eliminated invading organisms, and in preventing autoimmunity (Okeke and Uzonna, 2019).

T_{reg} has observed in the blood of the cancer mass and the draining lymph nodes of patients with different solid tumors (Bergmann et al., 2008; Whiteside, 2012). Many studies in humans and animal models (mice and rats) have proved that high numbers of T_{regs} in the tumor microenvironment are indicative of a poor prognosis. T_{reg} cells have to be thought that suppress tumor immunity, thus hindering the body's innate ability to control the growth of cancerous cells as colon carcinoma, renal cell carcinoma and ovarian carcinoma (Adeegbe and Nishikawa, 2013). There are two types of T_{reg} cells, natural (n) and adaptive (a) and differentiated by expanding CD127 expression which used as an excellent biomarker for (a) T_{reg} cells of many solid tumors as colon and ovary cancer (Whiteside, 2012).

Programmed cell death protein 1(PD-1) or CD279 is a member of extended CD28/CTLA-4 family of T cell regulators and expressed on the surface of activated T cells, B cells, and macrophages (Agata et al., 1996). Several studies in various types of human cancers have confirmed that tumors exploit PD-1- mediated immune suppression to escape immune surveillance (Bardhan et al., 2016). Members of the CD28/CTLA-4 family of co-stimulatory receptors and convey an inhibitory signal to the T cells and thus impede immune responses. PD-1 contributes to the immune tolerance of self-antigens by peripheral T cells. (Bour-Jordan et al., 2011). The present study aimed to analyze numbers of T regulatory cells in the peripheral blood of breast cancer patients and the expression of checkpoint molecule (PD-1) on immune T cells, and correlate the results with clinical status of the patients compared to healthy women.

Materials AND Methods

Ethics Committee

This study was approved by the Ethics Committee, Faculty of Medicine Tanta University, Egypt and executed in Centre of Excellence in Cancer Research (CECR), Tanta University Educational Hospital, Egypt with a Code number (3012/01/15).

Human subjects

The study included breast cancer patients (n=22). All patients were females (47 ± 10.48) years and range (31 – 68) years. The blood samples were collected from patients before surgery. The stages and grades of breast cancer were diagnosed histopathologicaly from carcinoma tissue samples taken after conservative surgery. The stages were divided according to the American Joint Committee on Cancer (AJCC) as follows: Six patients had stage II (27.2%), seven patients had stage III (31.8%) and nine patients had Stage IV (41%) as it has shown in Table 1. The patients were divided according to lymph nodes, five patients (22.7%) had from 1 to 3 axillary lymph nodes (pN1), eight patients (36.3%) had spread 4 to 9 axillary lymph nodes, or it has enlarged the internal mammary lymph nodes (pN2), and nine patients (41%) had spread to 10 or more axillary lymph nodes (pN3). The breast cancer patients were divided by grade, five patients (22.7%) had grade 1, seven patients (31.8%) had grade 2, and ten patients (45.5%) had grade 3 as shown in Table 1. The patients were divided according to histological subtypes as follows: most patients (n=15) had invasive ductal carcinoma (68.2%), four patients (18.2%) had invasive mammary carcinoma, two patients (9.1%) had lobular carcinoma, one patient (4.5%) had papillary carcinoma, as it has shown in Table 1. These different cases were compared with healthy volunteers (n=10) with mean age (31.75 ± 6.02) years and range (24-37) years.

Reagents

10X red blood cells (RBC) lysis Buffer (Multi-species, Catalog No. 00-4300). Before using, 10X RBC lysis buffer was diluted to 1X by adding 1 part phosphate buffer saline (PBS) (Catalog No. 557885) with 9 parts distilled water at room temperature.

Monoclonal antibodies

The mAbs used for surface cell staining in this study were purchased from BD Pharming™, BD Biosciences (USA). These mAbs included PE:-CD4 (Catalog No. 555347), V450: CD25 (Catalog No. 560355), APC-CD127 (Catalog No. 565185), and FITC-CD279 (PD-1) (Catalog No. 557860).

Equipment

Hematology Analyzer, Mindray BC-2800 manufactured by Mindray Ltd, (UK). Becton Dickinson Canto II Flow Cytometry (BD FACS Canto II), manufactured by BD Bioscience, (USA).

Table 1. Basic demographic data for breast cancer patients (n=22) at different stages (II, III, and IV) according to American Joint Committee on Cancer (Sobin and Wittekind, 2002).

Tumor Stage	Number (n)	Percentages (%)
Stage II	6	27.2
Stage III	7	31.8
Stage IV	9	41
Lymph node involvement		
pN1	5	22.7
pN2	8	36.3
pN3	9	41
Tumor grade		
Grade 1	5	22.7
Grade 2	7	31.8
Grade 3	10	45.5
Histological subtypes		
Invasive ductal	15	68.2
Invasive mammary	4	18.2
Lobular	2	9.1
Papillary	1	4.5

Determination of complete blood picture

Five ml of peripheral blood (PB) were collected from breast cancer patients and healthy volunteers in ethylene diamine tetraacetic acid (EDTA) tubes as an anti-coagulant substance. Complete blood pictures (CBC) were measured within 1h after drawn to calculate percentages of Haemoglobin (Hb), RBCs, leucocytes count (WBCs) and their total and differential numbers. In addition, lymphocyte cell percentages and absolute numbers were calculated.

Processing of the whole blood samples

Processing whole blood samples had been performed through the protocol (BD Biosciences, 2011). Firstly, 100µl of whole blood were mixed with aliquots monoclonal antibodies (CD279 (PD1), CD4, CD25, CD 127) for surface staining of immune cells of peripheral blood. The samples contained whole blood and antibodies, were incubated at dark conditions for 30min then the samples were incubated for 15min at dark conditions after addition 2ml lysing solution (1X) to lyes (RBCs) then spin down the samples at 1500rpm in the centrifuge for 5min. The supernatant discarded, and the pellets were washed with 2ml phosphate buffer saline (PBS). The supernatant discarded, and the pellets were suspended in (PBS). The cells were washed twice, then resuspended in 500ml PBS, and then analyzed with a flow cytometer (BD FACS Canto II). 10,000 events were analyzed for each sample using Becton Dickinson's software and FlowJo software version Vx.0.7 on hp laptop.

The forwards- and sides-scatter (FSC and SSC) were used; the dead cells were ignored, and gates were used around lymphocytes to exclude tumor cells that were specified. The relative proportions of the phenotypic subsets were measured using 4-quadrant and histogram analysis to calculate percentages of cells with immunophenotypes $CD4^+$ and T_{reg} ($CD4^+CD25^+$) in (PB) at different stages (II, III, and IV) of breast cancer women compared with healthy volunteers. The present study estimated the expression of PD-1 on total cells of lymphocytes in (PB) at different stages (II, III, and IV) of breast cancer women and correlated these percentages with healthy volunteers.

Statistical analysis

Data are the means of 4 or 5 replicates. The data were expressed as mean ± SD. Comparison between groups was carried out using one-way ANOVA. If there was a significant difference between means, Tukey post hoc comparisons among different groups were performed. For all statistical tests, P value was considered statistically significant when < 0.05. Statistical analyses were performed using Minitab version 18, Minitab, LLC, USA).

RESULTS

Complete blood pictures of breast cancer patients

In Table 1, breast cancer stages were divided into stages (II, III, and IV), according to the American joint committee on cancer **(**Sobin and Wittekind, 2002). Complete blood pictures (CBC) showed total leucocyte counts and its differential at different stages of breast cancer patients (II, III, and IV) compared to healthy volunteers. Total leucocyte count of breast cancer patients showed a significant increase in stage II (9.6±0.90)*10^3/µl than stages III and IV, and healthy women (5.4 ±0.5)*10^3/µl. The Percentages of neutrophil increased in stage II and IV (68.7±4.05, 66.5±2.04) respectively, compared to healthy women 60.1±2.82) (Table 2). Although the percentage of neutrophil of stage III had (57.2±6.4), lower than the healthy individuals the standard deviation had the largest number compared to stage II, IV and healthy women. In Table 3, the absolute number of neutrophils significantly increased in stage II (6598±787) than stage III and IV (3189±619, 3873±577) and healthy women (3267±399). The percentages of lymphocyte cells decreased in stage II and IV (22.3±2.57, 22.9±1.95) respectively, compared to healthy individuals (32.7±1.7), while the percentage of lymphocytes of stage III was close to healthy women (Table 2).

In Table 3, the absolute number of lymphocyte cells significantly decreased in stage, IV (1328±142) than stages III and II and healthy individuals. In Table 2, the monocyte percentages significantly increased in stage IV (6.7±1.51) than healthy women (4.75±0.96). In table (3), the absolute number of monocyte cells significantly increased in stage II and IV (635±140, 438±57) respectively than healthy individuals (255±35). In Table 2, the percentages of eosinophil cells significantly decreased in stage III (1.4±0.4) than healthy women. The percentages of basophil cells in stages (II and IV) did not change compared to healthy women. In Table 3, the absolute number of eosinophil cells increased in stage II (176±30) than healthy individuals (111±8). Whereas, the absolute number of eosinophils significantly decreased in stage III compared to healthy individuals. In Basophils, there is no significant differences among groups neither percentage nor numbers.

Table 2. The percentages of WBCs with differential at different stages (II, III, and IV) of breast cancer patients Compared to healthy individuals. The means with the same small letter are not significantly different.

Subjects	WBC 10^3/ul	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
Control	5.43±0.5^{b}	60.1±2.82^{b,c}	32.7±1.7^{a}	4.75±0.96^{b}	2.1±35^{a}	0.4±0.14
Stage II	9.6±0.90^{a}	68.7±4.05^{a}	22.3±2.57^{b}	6.7±1.51^{a,b}	1.8±0.31^{a,b}	0.32±0.09
Stage III	5.7±0.70^{b}	57.2±6.4^{c}	34.5±5.2^{a}	6.5±1.2^{a,b}	1.4±0.4^{b}	0.4±0.1
Stage IV	5.81±0.73^{b}	66.5±2.04^{a,b}	22.9±1.95^{b}	7.62±1.18^{a}	2.2±0.54^{a}	0.4±0.14
F-Value	38.34	7.85	19.0	3.98	4.58	0.78
P-Value	0.0001	0.003	0.0001	0.03	0.019	0.528

Table 3. The absolute numbers of WBCs differential cells at different stages (II, III and IV) of breast cancer patients compared to healthy individuals. The means with the same small letter are not significantly different.

Subjects	Neutrophils Abs.	Lymphocytes Abs.	Monocytes Abs.	Eosinophils. Abs.	Basophils Abs.
Control	3267±399^{b}	1860±206^{a}	255±22^{c}	111±8^{c}	22±6
Stage II	6598±787^{a}	2126±202^{a}	636±65^{a}	176±30^{a}	29±8
Stage III	3189±620^{b}	1943±80^{a}	365±28^{b,c}	76±21^{b}	26±6
Stage IV	3873±577^{b}	1328±143^{b}	438±57^{b}	127±22^{a,c}	23±6
F-Value	31.27	20.27	16.68	14.81	0.81
P-Value	0.0001	0.0001	0.0001	0.0001	0.512

Immunophenotyping of mononuclear cells isolated from peripheral blood of breast cancer patients

Flow cytometer analysis was performed on peripheral blood from healthy women included (n=10) and different stages (II, III and IV) of B.C. patients (n=22). In a previous study, the forwards and sides scattered were carried on dead cells and were excluded, the proportions of positive cells were calculated. Gates were applied on lymphocyte cells of healthy individuals and breast cancer patients (Figure 1). The percentages of the cells with phenotypes ($CD4^+$ and $CD4^+CD25^+$) were calculated as it has shown (Figures 2 and 3).

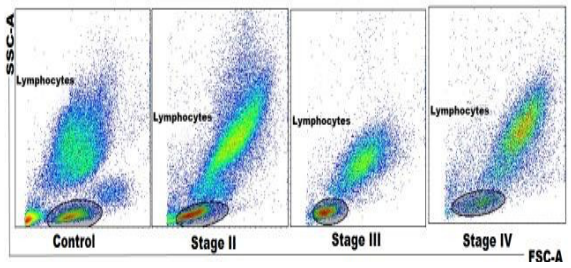

Figure 1. Represented flow cytometric data showed forwards and sides scattered for WBCs of healthy individuals and breast cancer patients at different stages (II, III, and IV), Gates performed on lymphocytes subpopulation.

In Table 4, the percentages of lymphocyte cells with immunophenotypes $CD4^+$ significantly decreased in stage II, III and IV, respectively, compared to $CD4^+$ cells of healthy individuals (56.1±4.63) %. The lowest percentage was in stage IV (33.66±3.61) % as it has shown in Figure 2. In Table 4, the percentages of T_{reg} ($CD4^+CD25^+$) at different stages (II, III and IV) of B.C. patients significantly increased with poor prognosis compared to healthy individuals. T_{reg} in stage IV of B.C. patients had higher percentage (7.91±1.09) % than stages III, II (6.48±1.02, 5.55±0.49) %, respectively, compared to healthy individuals (3.66±0.63) as shown Figure 3.

Expressions of PD-1 on immune cells with immunophenotypes $CD4^+$ and Treg ($CD4^+$ $CD25^+$)

In Table 4, the expression of PD-1 on immune cells with phenotypes $CD4^+$ significantly increased with poor prognosis in stages (III and IV), respectively (22.28±2.34, 24.33±2.75) compared to PD-1 expression on $CD4^+$ of healthy individuals (6.01±0.59) % as shown in Figure 2.

The expression of PD-1 on T_{reg} significant increase in stages II, III (25.47±1.36, 30.08 ±2.49), respectively, and the highest expression on T_{reg} was in stage IV (44.3±3.44) compared to PD-1 expression on T_{reg} of healthy individuals (19.05±1.96).

Table 4: Percentages of $CD4^+$ and T_{reg} cells ($CD4^+CD25^+$) and the percentages of PD-1 expressions on $CD4^+$ and T_{reg} cells.

Subjects	CD4 %	PD1/CD4 %	Treg %	PD1/Treg %
Control	$56.1±4.63^a$	$6.09±0.67^c$	$3.66±0.63^c$	$19.05±1.96^c$
Stage II	$40.68±5.34^b$	$13.31±3.09^c$	$5.55±0.49^b$	$25.87±1.66^b$
Stage III	$36.2±4.39^b$	$22.3±2.34^a$	$6.48±1.02^{a,b}$	$30.1±2.49^b$
Stage IV	$33.6±3.61^b$	$24.33±2.75^a$	$7.91±1.09^a$	$44.3±3.44^a$
F-Value	20.59	52.56	19.54	72.06
P-Value	0.0001	0.0001	0.0001	0.0001

In Table 5, the absolute numbers of lymphocyte cells with immunophenotypes $CD4^+$ cells significantly decreased in breast cancer patients in stages to III (725±103, 455±51) respectively, compared to healthy women (1047±173). In Table 5, the absolute number of lymphocyte cells with immunophenotypes ($CD4^+CD25^+$) T_{reg} cells increased in breast cancer patients from stage II to stage III (123±17, 125±21), respectively, compared to healthy individuals (67±10), Although the number of T_{regs} in stage IV (101±12) lower than stage II, III.

Expression of CD127 on Treg ($CD4^+$ $CD25^+$):

In Table 6, the surface marker CD127 expressed with low levels on T reg of healthy individuals (12.9±2.6)% and this percentage significantly decreased at different stages of breast cancer patients from stage II (6.13±2.1)% to stage III (3.56±1.7)%, the lowest percentage was in stage IV (1.81±0.9).

Table 5: The absolute numbers of $CD4^+$ and T_{reg} cells and their level of expression of PD-1.

Subjects	CD4 Abs.	PD1/CD4 Abs.	Tregs Abs.	PD1/Treg Abs.
Control	$1047±173^a$	$63±7^c$	$67±10^c$	$13±2^c$
Stage II	$858±93^{a,b}$	$105±10^b$	$123±17^a$	$31±4^b$
Stage III	$725±103^b$	$159±12^a$	$125±21^a$	$38±6^a$
Stage IV	$455±51^c$	$110±21^b$	$101±12^b$	$45±7^a$
F-Value	24.6	25.9	11.25	27.69
P-Value	0.0001	0.0001	0.001	0.0001

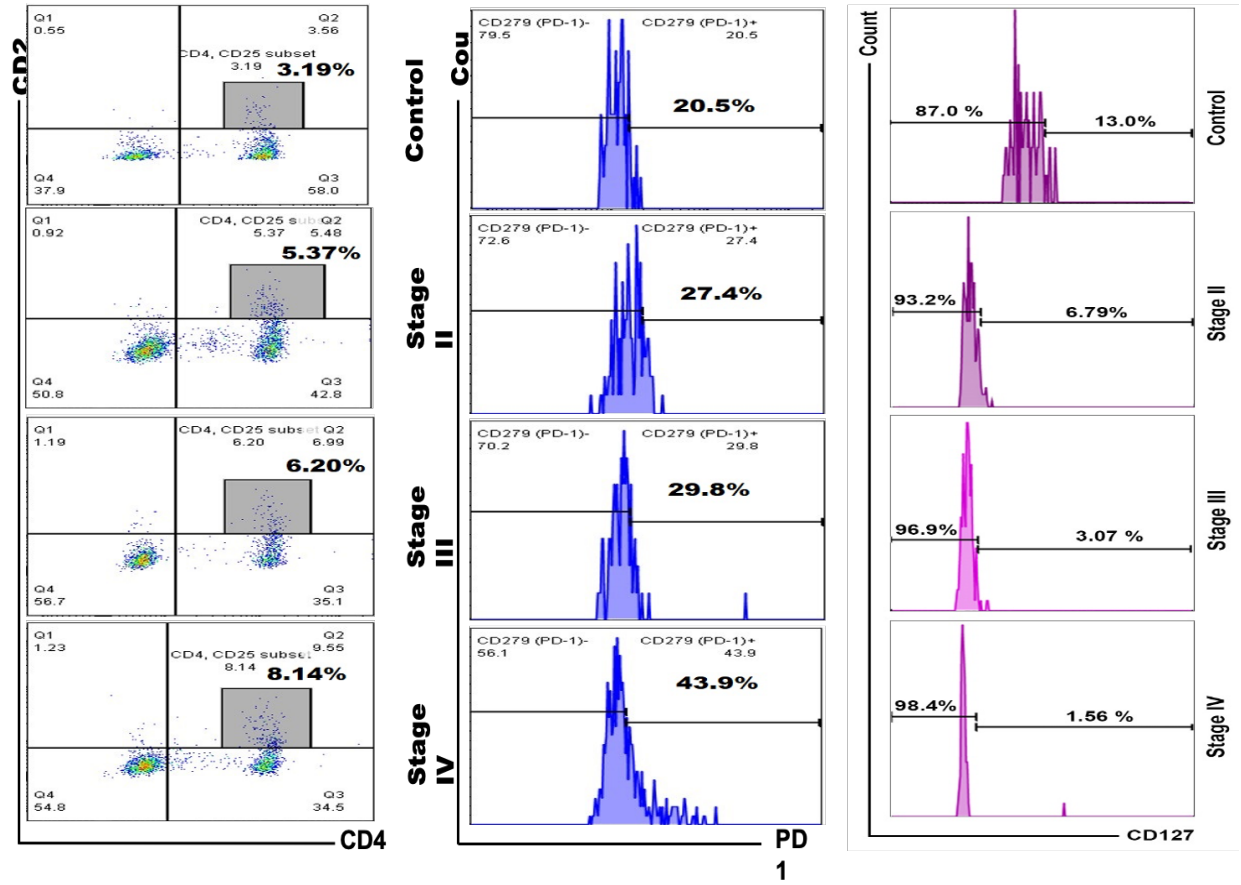

Figure 3: Flow cytometry data for immunophenotypes cells Tregs ($CD4^+CD25^+$) of lymphocyte cells in breast cancer patients at different stages (II, III, and IV) compared to healthy individual (A). Flow cytometry data for PD-1(CD279) expressions on Tregs of breast cancer patients at different stages (II, III, and IV) compared to healthy individual (B). Flow cytometric analysis of (CD127) expression on Tregs of breast cancer patients at different stages (II, III and IV) compared to healthy individuals (C).

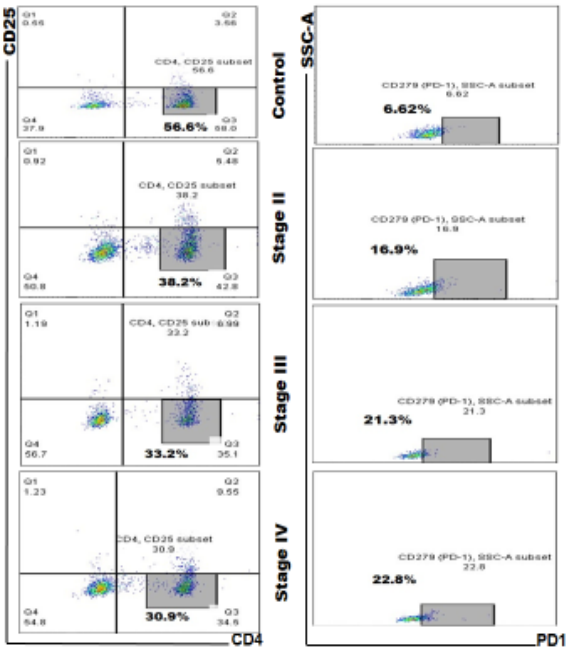

Figure 2: Immunophenotyping of $CD4^+$ cells in breast cancer patients at different stages (II, III, and IV) compared to healthy individuals (A). Flow cytometry data for PD- 1(CD279) expression on $CD4^+$ cells of breast cancer patients at different stages (II, III, and IV) compared to healthy individuals (B).

Table 6. The percentages of $CD127^+$ expressions on T_{reg} cells at different stages (II, III, and IV) of breast cancer patients compared to healthy women.

Subjects	Treg($CD4^+$ $CD25^+$)	$CD127^+$/ Tregs
Control	3.66 ± 0.63^c	12.9 ± 2.6^a
Stage II	5.55 ± 0.49^b	6.51 ± 2.6^b
Stage III	$6.48 \pm 1.02^{a,b}$	3.56 ± 1.7^c
Stage IV	7.91 ± 1.09^a	1.81 ± 0.9^c
P-Value	0.0001	0.0001

DISCUSSION

Breast cancer is the most common cancer that leads to death among women accounting 38.8% of cancer-diagnosed woman's **(**Ibrahim *et al.,* 2014). In this study, breast cancer diagnosis occurs in later stages during which most women agonize symptoms of breast cancer. Cancer in late stages has aggressive features and poor prognosis (III and IV); when occurs in young ages causes high mortality. Our data are in agreement with Bowen *et al.* (2006) and Lund and Dumeaux (2008).

These authors reported that younger women and younger African-American women, in particular, are more likely to present a breast cancer diagnosis with more aggressive and associated with higher mortality.

The present study showed significant differences in total white blood cells (WBCs) and their differentials in breast cancer patients compared to healthy women. In early stage, II WBCs count increased compared to stages III and IV, due to the increase of neutrophil cells as a result of a strong response of the immune system to cancer compared to neutrophil cells of late stages (III and IV) of breast cancer and healthy volunteers. Our data are similar to the results of Kamphorsta *et al.* (2016), who showed a high WBCs count, linked to the recurrence risk of early-stage breast cancer. The present study showed an increase in monocyte cells in breast cancer patients compared to healthy volunteers. These clarify the important role of this cell type to fight cancer. Our data are compatible with Zhang *et al.* (2017) who showed that the high frequencies of circulating monocytes serve as a diagnostic biomarker in breast cancer and has a potential role in reflecting breast cancer progression.

We found a significant correlation between the frequencies of $CD4^+$ cells and different stages of the breast cancer; i.e. as cancer stage increased from stage II to late-stage III and IV, the percentage of $CD4^+$ decreased in the peripheral blood compared to the percentage in peripheral blood of healthy women. This decrease may be due to the migration of these cells in cancer tissue of breast or these surface markers conjugated with another surface marker of tumor cells. Our data agreed with Bergmann *et al.* (2007) who showed a significant correlation between the frequencies of both $CD4^+$ and $CD8^+$ T cells in the tumor tissues and the size of the tumors. In the current, we demonstrated that percentages of T_{reg} cells had a highly significant increase with stages of progression compared to healthy volunteers.

The percentage increased from stage II to stage III and the highest proportion was observed in stage IV; this means high percentages is related to poor prognosis of breast cancer and low survivor.

T_{reg} cells have a role in the suppression of immune cells which preventing immune escape. Our data are consistent with Bettelli *et al.* (2006) and Curiel (2007). They reported that T_{reg} has an important role in modulation the immune cells and keep tolerance to self-antigens, so it has an important role in immunity. It prevents autoimmune disease, and it suppresses induction and proliferation of effector T cells. The surface markers of T_{reg} which differentiated from other immune cells are $CD4^+$, $CD25^+$ and $FOXP^+$, but they limited T_{reg} consisted on $FOXP3^+$, this surface marker present in normal and adaptive T_{reg} cells.

Our study used CD127 as a surface marker to differentiate between normal T_{reg} and adaptive T_{reg} cells. Our data showed adaptive T_{reg} cells raised in the blood of breast cancer patients. Patients compared to healthy volunteers'. The percentage of adaptive T_{reg} cells increased with late stages, the highest percentage of adaptive T_{reg} cells was in stage IV of breast cancer patients. Our data showed CD127 expressed on normal T_{reg} cells with low levels, this low expression decreased with adaptive T_{reg} cells raised in the blood of breast cancer.

Results agreed with Weihong *et al.* (2006), who reported that many solid tumor as colon and lung cancers used expression of surface marker CD127 as a useful marker for identification of T reg cells ($CD4^+$ $CD25^+$) in peripheral blood of humans. Our data in agreement with Whiteside (2012), who reported that inducible T_{reg} cells or adaptive T_{reg} cells are the major subset of T_{reg} present in the blood of cancer patients. These adaptive T_{reg} cells are different phenotypically and functionally from $FOXP3^+$ natural normal T_{reg} cells which responsible for peripheral tolerance. But adaptive T_{reg} cells mediate powerful suppression of effector T cells via diverse mechanisms through producing immunosuppressive cytokines, Whiteside (2012), did not explore why (a) T_{reg} raised and consist on cytokines which raising in the blood for many causes including inflammation and cancer.

Our data showed significantly higher expression levels in the percentage of PD-1 expression on immune T cells, including immune cells with phenotype $CD4^+$ and $CD4^+CD25^+$. Our data showed the important role of PD-1 expression.

Furthermore, the high percentage of PD-1 expression on both CD4 and T_{reg} correlated with poor prognosis and the highest percentage of expression in late stages (III and IV) had an important role in the suppression of mediated immune cells as $CD4^+$ cells, which decrease from stage II to late stages III and the lowest percentage in stage IV. This converse proportion between mediated immune cells CD4 and immune checkpoint molecule PD-1 lead to low survivor to patients. Our data agreed with Seidel et al. (2018), who reported that checkpoints molecules (PD-1/PD-L1 and CLTA- 4), are highly expressed, allowing the complete blockade of immune cells and outgrowth of cancer cells. Our data similarly with Simon *et al.* (2016), who reported PD-1 suppresses T cell activation and promotes tumor cell immune escape and cancer progression.

CONCLUSION

Detection of T regulatory cell levels and expression of checkpoint molecule PD-1 (CD279) on immune T cell of peripheral blood of breast cancer patients help us to understand the biology of the disease and can lead to the application of anti-checkpoint molecules in the treatment of cancer patients in Egypt.

CONFLICT OF INTEREST

The authors claim no conflict of interest.

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EACR is an NGO society that was declared by the Ministry of Social Solidarity (Egypt) No. 1938 in 19/11/2014 based on the initiative of Prof. Mohamed Labib Salem, the current Chairman of EACR. EACR aims primarily to assist researchers, in particular young researchers in the field of cancer research through workshops, seminars and conferences. Its first international annual conference entitled "Anti-Cancer Drug Discovery" was successfully organized in April 2019 (http://acdd.tanta.edu.eg). Additionally, EACR aims to raise the awareness of the society about the importance of scientific research in the field of cancer research in prediction, early diagnosis and treatment of cancer. EACR is also keen to outreach the scientific community with periodicals and news on cancer research including peer-reviewed scientific journals for the publication of cutting-edge research. The official scientific journal of EACR is "International Journal of Cancer and biomedical Research (IJCBR: https://jcbr.journals.ekb.eg) was successfully issued in 2017 and has been sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

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