

## Original Research Article

# Evaluation of immune status in patients with breast cancer in Sokoto, Northwestern, Nigeria

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### ABSTRACT

**Background:** Breast cancer is a major public health problem especially in Sub-Saharan Africa, Nigeria inclusive. It is a leading cause of cancer-related deaths worldwide. The present study assessed the immune status of breast cancer patients using CD4<sup>+</sup> T cell count, CD8<sup>+</sup> T cell count, CD4<sup>+</sup>/CD8<sup>+</sup> ratio and some leucocytes.

**Methods:** A total of sixty nine (69) subjects that comprised of forty six (46) breast cancer patients attending the Breast Clinic of Usmanu Danfodiyo University Teaching Hospital, Sokoto and twenty three (23) age- and gender-matched apparently healthy individuals as controls were included in the study.

**Results:** The CD4<sup>+</sup> T cells (cell/  $\mu$ l) and CD8<sup>+</sup> T cells (cell/  $\mu$ l) were enumerated using Flow Cytometric method, while CD4/CD8 ratio was calculated from the values obtained from the CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells. Even though, there were no significant differences in CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and CD4<sup>+</sup>/CD8<sup>+</sup> ratio based on breast cancer stages and therapy types.

**Conclusions:** It is hereby concluded that, the monocyte counts and CD4<sup>+</sup>/CD8<sup>+</sup> ratio were higher in breast cancer patients and this could be used to differentiate the breast cancer patients from the normal control subjects.

**Keywords:** Breast cancer, CD4<sup>+</sup>T Cells, CD8<sup>+</sup>T Cells, CD4<sup>+</sup>/CD8<sup>+</sup> ratio, Leucocytes, Sokoto

### INTRODUCTION

Breast cancer is a major public health problem especially in Sub-Saharan Africa, Nigeria inclusive. It is a leading cause of cancer related deaths worldwide.<sup>1,2</sup> The present study assessed the immune status of breast cancer patients using CD4<sup>+</sup> T cell count, CD8<sup>+</sup> T cell count, CD4<sup>+</sup>/CD8<sup>+</sup> ratio and some leucocytes. It is estimated that worldwide over 50, 8000 women died in 2011 due to breast cancer.<sup>3</sup>

Breast cancer is the leading cause of cancer death among females accounting for 23% (1.38 million) of the total cancer cases and 14% (458,400) of the cancer deaths in women.<sup>4,5</sup> Breast cancer is a major public health problem

especially in Sub-Saharan Africa, Nigeria inclusive. It is a leading cause of cancer-related deaths worldwide. The present study assessed the immune status of breast cancer patients using CD4<sup>+</sup> T cell count, CD8<sup>+</sup> T cell count, CD4<sup>+</sup>/CD8<sup>+</sup> ratio and some leucocytes.<sup>6,7</sup> Since, breast cancer is one of the most common and frequently diagnosed malignant in Nigeria and is the second principal cause of death among women worldwide.<sup>8</sup>

Previous studies of breast cancer in humans in various populations have shown an increase in morbidity and mortality of the breast cancer patients. The increasing global incidence of breast cancer also emphasizes the need to understand the various mechanisms involved in breast tumorigenesis, hence the need to assess the

immune status of breast cancer patients in Sokoto. Evaluation of immunological parameters may be beneficial for assessing the immune functions in patients with breast cancer attending Specialists Hospital, Sokoto. Result from this study apart from adding to knowledge, will provide valuable information that will aid in the management of breast cancer patients in this environment.

The aim of this study is basically to evaluate the immune status in patients with breast cancer in Sokoto, Nigeria, and to enumerate the CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts in breast cancer patients and controls, to correlate the leucocyte count of breast cancer patients with that of controls, compare the plasma levels of Immunological and Hematological parameters in breast cancer patients based on staging of cancer as well as to compare the plasma levels of Immunological and Haematological parameters in breast cancer patients based on types of therapy.

**METHODS**

A total of sixty nine (69) subjects that comprised of forty six (46) breast cancer patients and twenty three (23) age- and sex-matched apparently healthy individuals as controls were included in this study. The CD4<sup>+</sup> T cells (cell/ µl) and CD8<sup>+</sup> T cells (cell/ µl) were enumerated using Flow Cytometric method, while CD4/CD8 ratio was calculated from the values obtained from the CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells.

Differential leucocytes counts were enumerated using Sysmex haematology analyzer. Five millilitres of (5 ml) of blood were collected from each of the consenting participants in EDTA vacutainer blood specimen container. The data obtained was analysed using Graph pad InStat<sup>®</sup> and SPSS Version 20.

The results were expressed as mean±standard error of mean and presented in tabular and figures. Results CD4<sup>+</sup> cell, CD8<sup>+</sup> cell and differential leukocyte counts obtained from breast cancer patients were compared with those of controls using paired two-tailed.

**Inclusion and exclusion criteria for patients and control**

Histological confirmed breast cancer patients that have not started treatment and those that started treatment for the tumour were included in this study.

Breast cancer patients of 18 years and above were included in this study while those that are less than 18 years were excluded. Breast cancer patients that are cigarette smokers and those that have diabetes, liver or renal dysfunction were excluded from the study. Age- and sex- matched apparently healthy subjects were included in the study.

**RESULTS**

Comparison of Haematological parameters in Breast Cancer Patients with controls differ significantly at P<0.05 as shown in Table 1. Immunological Parameters in breast cancer patients and controls differ significantly from controls at p<0.01 as in Table 2.

Similarly, Immunological and Haematological Parameters based on Cancer Staging values are not statistically significant (p>0.05) using ANOVA and based on types of therapy values are not statistically significant (p>0.05) using student t-test as shown table 3 and 4 respectively. Table 5 shows Correlation Coefficient (r) between haematological parameters among breast cancer patients values with asteric differ significantly at \*(p<0.05).

**Table 1: Haematological parameters in breast cancer patients and controls.**

Parameters	Controls (n=23)	Breast cancer patients (n=46)	P value
Neutrophil (%)	43.48±1.74	42.48±3.16	0.764
Lymphocyte (%)	53.15±1.75	51.35±3.31	0.597
Eosinophil (%)	1.85±0.32	2.91±0.62	0.097
Monocyte (%)	1.43±0.39	3.65±0.69*	0.040
Basophil (%)	0.02±0.02	0.00±0.00	0.484

Values are Mean±SEM, n= number of subjects, values bearing asteric (\*) differ significantly from controls at p<0.05.

**Table 2: Immunological parameters in breast cancer patients and controls.**

Parameters	Controls (n=23)	Breast cancer patients (n=46)	P value
CD4+ (µl/count)	412.43±28.39	454.02±28.86	0.365
CD8+ (µl/count)	280.87±18.37	267.11±17.18	0.619
CD4+/CD8+ Ratio	1.44±0.06	1.70±0.06**	0.007

Values are mean ± SEM; n= number of subjects; CD4= cluster of differentiation type 4; CD8= cluster of differentiation type 8, values bearing asteric (\*\*) differ significantly from controls at p<0.01.

**Table 3: Immunological and haematological parameters based on cancer staging.**

Parameters	TNM staging				P value
	Stage 1	Stage 2	Stage 3	Stage 4	
Neutrophil (%)	50.50±6.50	42.74±1.99	47.38±4.81	33.50±2.50	0.38
Lymphocyte (%)	47.00±5.00	54.00±2.01	47.75±4.42	65.00±4.00	0.22
Eosinophil (%)	2.00±2.00	2.06±0.38	1.38±0.82	0.00±0.00	0.56
Monocyte (%)	0.50±0.50	1.00±0.38	3.50±1.31	1.50±1.50	0.98
Basophil (%)	0.00±0.00	0.03±0.03	0.00±0.00	0.00±0.00	0.95
CD4+ (cell/µl)	223.50±91.50	446.00±33.34	564.13±61.95	380.50±121.50	0.13
CD8+ (cell/µl)	138.50±34.50	262.15±20.15	321.50±40.04	262.50±54.50	0.24
CD4+/CD8+ Ratio	1.50±0.30	1.71±0.07	1.79 ±0.11	1.35±0.15	0.48

Values are Mean±SEM, TNM= Tumour Node Metastasis, values are not statistically significant (p>0.05) using ANOVA.

**Table 4: Immunological and haematological parameters based on types of therapy.**

Parameters	Types of therapy		P value
	Radiotherapy (n=31)	Chemotherapy (n=15)	
Neutrophil (%)	42.90±2.09	44.67±3.21	0.64
Lymphocyte (%)	53.84±2.08	51.73±3.28	0.58
Eosinophil (%)	2.13±0.42	1.27±0.47	0.21
Monocyte (%)	1.00±0.38	2.33±0.85	0.11
Basophil (%)	0.30±0.32	0.20±0.10	0.49
CD4+ (cell/µl)	448.71±36.98	465.00±46.29	0.79
CD8+ (cell/µl)	259.23±20.36	283.40±32.32	0.52
CD4+/CD8+Ratio	1.70±0.76	1.69±0.09	0.94

Values are Mean± standard error of mean, n= number of subjects on therapy, CD4= cluster of differentiation type 4; CD8= cluster of differentiation type 8; values are not statistically significant (p>0.05) using student t-test.

**Table 5: Correlation coefficient (r) between haematological parameters among breast cancer patients.**

Haematological parameters	Immunological parameters		
	CD4+ (cell/µl)	CD8+ (cell/µl)	CD4+/CD8+Ratio
Neutrophil (%)	r = 0.343* p = 0.020	r = 0.338* p = 0.021	r = 0.089 p = 0.555
Lymphocyte (%)	r = -0.290 p = 0.051	r = -0.277 p = 0.062	r = -0.116 p = 0.444
Eosinophil (%)	r = -0.211 p = 0.159	r = -0.254 p = 0.088	r = -0.088 p = 0.561
Monocyte (%)	r = -0.062 p = 0.684	r = -0.072 p = 0.635	r = 0.065 p = 0.668
Basophil (%)	r = 0.050 p = 0.741	r = 0.070 p = 0.645	r = -0.038 p = 0.062

Values are correlation coefficient (r) and p values (p); CD4= cluster of differentiation type 4; CD8= cluster of differentiation type 8, values with asteric differ significantly at \*(p<0.05).

## DISCUSSION

A total of sixty nine (69) subjects that comprised of forty six (46) breast cancer patients attending the Breast Clinic of Usmanu Danfodiyo University Teaching Hospital, Sokoto and twenty three (23) age- and gender-matched apparently healthy individuals as controls were included in the study. The CD4<sup>+</sup> T cells (cell/ µl) and CD8<sup>+</sup> T cells (cell/ µl) were enumerated using Flow Cytometric method, while CD4/CD8 ratio was calculated from the

values obtained from the CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells. Differential leucocytes counts were enumerated using Sysmex haematology analyzer. Majority of the breast cancer patients were married (95.7%), 89.1% had no previous breast cancer disease, while 67.4% were currently on radiotherapy.

The mean age of the patients was 39.94±1.70 years. Monocyte count in breast cancer patients (3.65±0.69%) was significantly higher (p<0.05) than the corresponding values in controls (1.43±0.39%). However, no significant

differences were observed for neutrophil (%), lymphocyte (%), eosinophil (%), and basophil (%) between breast cancer patients and controls. CD4<sup>+</sup>/CD8<sup>+</sup> ratio in breast cancer patients (1.70±0.06) was significantly higher ( $p<0.05$ ) than the corresponding values in controls (1.44±0.06).

No significant differences were observed for CD4<sup>+</sup> and CD8<sup>+</sup> between breast cancer patients and controls. There were significant positive correlations between neutrophil% and CD4<sup>+</sup> ( $r=0.343$ ,  $p=0.020$ ) and CD8<sup>+</sup> ( $r=0.338$ ,  $p=0.021$ ), while neutrophil% and CD4<sup>+</sup>/CD8<sup>+</sup> ratio were not correlated. The CD4<sup>+</sup>/CD8<sup>+</sup> Ratio and monocytes were significantly higher ( $p<0.05$ ) in the breast cancer patients, while the levels of the CD4<sup>+</sup> and CD8<sup>+</sup> counts were similar in the breast cancer patients and controls. There were no significant differences in CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and CD4<sup>+</sup>/CD8<sup>+</sup> ratio based on breast cancer stages and therapy types. It is hereby concluded that, the monocyte counts and CD4<sup>+</sup>/CD8<sup>+</sup> ratio were higher in breast cancer patients and this could be used to differentiate the breast cancer patients from the normal control subjects.

However, it was observed that majority of the breast cancer patients are married (95.7%), most of them within the age group of 41-50 years (32.6%). 89.1% of the subjects have no previous history of breast cancer and sixty seven percent (67%) were correctly on radiotherapy. The monocyte count was significantly higher in breast cancer patients compared with controls. This is because monocytes (macrophages) are known to preferentially lyse tumour cells.

Other haematological indexes, including neutrophil, eosinophil, basophil and lymphocyte in breast cancer patients were not different from the values obtained in controls. This is in contrast to the previous studies by who reported that the significant differences were seen in haematological indexes between breast cancer patients and controls.<sup>2</sup> The differences observed between the present study and that of could be due to methodology used, the haematological parameters were counted using manual method while that of was automated using automated machine (Sysmex KX-21N).<sup>2</sup>

The biologic mechanisms leading to the development of breast cancer are not clearly understood, but the role of immunity and carcinogenesis has been well established. In the present study, though not statistically significant, the CD4<sup>+</sup> cell count in breast cancer patients was higher than in controls. This is in agreement with previous studies, who reported a significant increase in CD4<sup>+</sup> cell count in comparison with the controls.<sup>4,6</sup>

Also reported that CD4<sup>+</sup> cell count was significantly higher in breast cancer patients. However, the finding of this study is in contrast to the studies conducted by, who reported a significant decrease CD4<sup>+</sup> cell count in breast cancer patients.<sup>7,9</sup> Also reported that there was significant

depletion in the CD4<sup>+</sup> cell count in breast cancer patients. The lower CD8<sup>+</sup> cell count in breast cancer patients observed in this study was in agreement with the previous researches by and, who reported that there was decreased in number of CD8<sup>+</sup> cell count compared with controls. The frequency of CD8<sup>+</sup>T cells undergoing spontaneous apoptosis in the blood of patients with cancer was found to be significantly elevated relative to that in sex- or age-matched healthy controls.<sup>3,6,9</sup> CD8<sup>+</sup> T cells were preferentially targeted for cell death compared to circulating CD4<sup>+</sup> T cells.<sup>10</sup>

The implication of the significant decrease of CD8<sup>+</sup> T cells in breast cancer subject is that it may lead to reduced survival in breast cancer subjects since the CD8<sup>+</sup> T cells were preferentially targeted for cell death compared to circulating CD4<sup>+</sup> T cells according to and.<sup>3,10</sup> The CD4<sup>+</sup>/CD8<sup>+</sup> ratio significantly increased in breast cancer patients compared to controls. This is in contrast the studies of who reported no significant difference in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio.<sup>9</sup> The increased CD4<sup>+</sup>/CD8<sup>+</sup> ratio observed in the present study could be due to an increased CD4<sup>+</sup> cell count in response to an increased demand for CD8<sup>+</sup> that is used for Cytotoxic-mediated killing of cancerous cells.

The breast cancer staging appear not be an important factor in regulating the blood levels of immunological and haematological parameters. The staging of breast cancer was done using TNM (tumour node metastasis) staging system which was carried out by Consultant Surgeons in the clinic. There were no significant differences ( $P<0.05$ ) in CD4<sup>+</sup>, CD8<sup>+</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> ratio and the haematological parameters (neutrophil %, lymphocyte %, eosinophil %, monocyte %, basophil %) based on staging. There were positive and negative correlations between the immunological and haematological parameters, with neutrophil being significantly correlated with CD4<sup>+</sup> and CD8<sup>+</sup> cell counts.

## CONCLUSION

It is hereby concluded that, the levels of plasma CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and CD4<sup>+</sup>/CD8<sup>+</sup> ratio were not different according to breast cancer stages and therapy types. However, the monocyte count and CD4<sup>+</sup>/CD8<sup>+</sup> ratio were higher in breast cancer patients and this could be used to differentiate the breast cancer patients from the normal control subjects.

## Recommendation

Considering the findings of the present study, it could be recommended that: Immunological and Haematological parameters should be assessed periodically in breast cancer patients and this could be useful in the diagnosis and management of breast cancer patients. Further studies are necessary to focus on the role and mechanisms of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and inflammatory cytokines in breast cancer development and progression.

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