ASSESSMENT OF IMMUNE STABILITY IN BREAST CANCER SUBJECTS

Oluboyo, A.O Dr.
Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe, University, Nnewi Campus, Anambra State, Nigeria
Meludu S.C, Prof.
Department of Human Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
Onyenekwe C.C, Prof.
Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe, University, Nnewi Campus, Anambra State, Nigeria
Oluboyo, B.O, Dr.
Molecular Diagnostic Laboratory, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria
Chianakwanam, G.U, Prof.
Emegakor, C, Dr.
Surgical Out patients' Department, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria

Abstract

Cancer is a cause of death worldwide and the socio-economic impact of cancer is enormous and disturbing. Breast cancer is becoming a significant health concern for women across Nigeria and immune instability may be responsible. Therefore, the present study was designed to assess the immune stability of breast cancer subjects using CD4 + T cell, CD8 + T cell counts and the CD4/CD8 ratio. One hundred subjects were randomly recruited for the study and grouped into Breast cancer subjects (BCA) (n=50) and control (n=50). CD4 + T cells (µl/count) and CD8 + T cells (µl/count) were estimated using flow cytometric method while CD4/CD8 ratio was calculated from the values obtained from the CD4 + T cells and CD8 + T cells. CD4+ T cells and CD8+ T cells showed significant decrease ($p<0.05$) in the breast cancer subjects compared to the control subjects. The

suppressed CD4 + and CD8 + T cell counts in breast cancer subjects may indicate immune instability in the cancer subjects.

Keywords : CD4 + T cells, CD8 + T cells, CD4/CD8 ratio, breast cancer

Introduction

Cancer has become an increasingly important public health problem in developing countries, including Africa (Farmer *et al*, 2010). As public and professional awareness of the cancer problem has grown, so has interest in the pattern of disease diagnosis and treatment outcome. Breast cancer is the most frequently diagnosed malignancy and the second principal cause of death among women worldwide as well as in Nigeria (Okobia *et al*, 2006). It 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 (Jemal *et al*, 2011).

It has also been shown that the development of cancer is associated with alterations in numbers and functions of immune cells in the peripheral circulation and especially at the sites of tumor progression (Whitefield, 2013). Antigen-specific immune responses result from a complex dynamic interplay between antigen presenting cells, T lymphocytes, and target cells (Scott et al, 2012). The mechanisms of tumor cell killing by antibodies can be due to direct cell killing, such as through receptor blockade or agonist activity, induction of apoptosis, or delivery of a drug, radiation, or cytotoxic agent; immune-mediated cell killing mechanisms; regulation of T cell function; and specific effects on tumor vasculature and stroma. McArdle *et al* (2004) stated that disease progression in cancer is not solely determined by the characteristics of the tumour but also by the host response). Liu et al (2012) reported that immune response may play an important role in cancer progression. Tumor-infiltrating lymphocytes (TILs) reflect a local immune response and could be a key mechanism in controlling tumor progression (Mahmoud et al, 2011, Mahmoud et al, 2012). Tumor-derived factors have been shown to induce death of immune cells at the tumor sites and in the peripheral circulation (Whiteside, 2010). The frequency of CD8+ 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 (Hoffmann *et al*., 2002). CD8+ T cells were preferentially targeted for cell death compared to circulating CD4+ T cells (Tsukishiro, 2003). Therefore, the study aimed to assess the immune stability of breast cancer subjects using CD4 + T cells, CD8 + T cell counts and the CD4/CD8 ratio.

Methods

Breast cancer subjects who were attending the general Surgery Department (Surgical Out Patients' Clinic) of the Nnamdi Azikiwe University Teaching Hospital (NAUTH) were investigated. Ethical clearance was obtained from the Ethics Committee of the Nnamdi Azikiwe University Teaching Hospital (NAUTH). A total of fifty breast cancer subjects were recruited using non probability consecutive sampling. The mean age of the breast cancer subjects was 43 ± 12 years. Twenty seven subjects were in stage 0, eighteen in stage 1 and five subjects were in stage 3 at the time they were recruited. A total number of twenty three subjects were on chemotherapy and they received four courses of combination therapy of Cyclophosphamide, (500mg/m2), Adriamycin (50mg/m2) and 5-Fluorouracil (500mg/m2). The drugs were given at intervals of three weeks.

A total of fifty apparently healthy females without any family history of cancer participated in the study. The mean age of the subjects was 40±10 years. They were recruited after they underwent self and clinical breast examination and no clinical tumour was observed. The subjects were apparently healthy subjects without any other diseased condition.

Two milliliters of blood samples were collected from each subjects into Disodium ethylenediaminetetraaceticacid (EDTA) for the estimation of CD4 + and CD8 + T cells within 2 hours of collection using flow cytometric method (Fryland, 2006).

The Mean and Standard Deviation (SD) were calculated for each parameter. Differences in the means for each parameter between the two groups were compared using Student's t test and analysis of variance (ANOVA).

Results

The results are presented in tables. The results show that CD4 + T cells and CD8 + T cells in the breast cancer subjects were significantly decreased ($p<0.05$) compare with the control subjects while the CD4/CD8 ratio show significant increase (Table 1). There was no significance difference in CD4 + T cells, CD8 + T cells and CD4/CD8 ratio based on stages and chemotherapy (Table 2 and 3).

Table 1 : Immune status in breast cancer subjects

Parameters	Breast cancer	Control	P value
CD4+ T cells (µl/count)	675± 100	1045± 322	0.000*
CD8 +T cells (µl/count)	364± 50	775 ±100	0.000*
CD4/CD8 Ratio	1.87 ±0.24	1.65 ±0.29	0000*

*significant at $p<0.05$

Table 2: Immune status in breast cancer according to stages

Parameters	Breast cancer subjects in stage 0 n=27	Breast cancer subjects in stage 1 n=18	Breast cancer subjects in stage 3 n=5	F-values	P-values
CD4+ T cells	693 ± 96	663 ± 102	624 ± 112	1.229	0.302

(µl/count)					
CD8 +T cells (µl/count)	371± 55	362 ± 44	337 ± 41	0.970	0.386
CD4/CD8 Ratio	1.89 ± 0.27	1.83 ± 0.17	1.86 ± 0.32	0.292	0.748

Table 3: Immune status in breast cancer subjects based on chemotherapy

Parameters	Breast cancer subjects not on chemotherapy N=27	Breast cancer subjects on chemotherapy N=23	P-values
CD4+ T cells (µl/count)	680 ± 93	670 ± 108	0.737
CD8 +T cells (µl/count)	362 ± 40	368 ± 61	0.691
CD4/CD8 Ratio	1.89 ± 0 .24	1.84± 0 .24	0.519

Discussion

The study observed significant decrease in the levels of CD4 + and CD8 + T cells in breast cancer subjects. Other studies have associated this finding with the spread of disease in patients with breast, malignant melanoma, lung, and colorectal cancers (Blake-Mortimer *et al*, 2004). The frequency of CD8+ T cells undergoing spontaneous apoptosis in the blood of patients with cancer was found to be significantly elevated relative to sex- or age-matched healthy controls (Hoffmann *et al*., 2002). This is because CD8+ T cells were preferentially targeted for cell death compared to circulating CD4+ T cells (Tsukishiro *et al*., 2003). Blake-Mortimer *et al* (2004) had related higher cytotoxic T lymphocytes (CTL) count to longer survival and that CTL count was significantly associated with other subsets, such as helper T cells which also play a crucial role in cell-mediated immunity. Liu *et al* (2012) has shown that tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment are predominantly CD8+ T cells and they produce interferon-gamma through interaction with tumor-related antigens, potentially leading to tumoricidal activity by induction of apoptosis and / or macrophage tumor killing activity. The implication of the significant decrease of CD8+ T cells in breast cancer subject is that it may lead to reduced survival in breast cancer subjects since the CD8+ T cells were preferentially targeted for cell death compared to circulating CD4+ T cells according to Tsukishiro *et al* (2003) and Blake-Mortimer *et al* (2004).

It was concluded that there was immune instability in the breast cancer subjects compared with the control subjects.

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