Evaluation of interleukin-6 in patients with breast cancer in Sokoto-Nigeria

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Abstract

Introduction: Breast cancer is cancer that develops from breast tissue, Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, nipple discharge, or a red, scaly patch on the skin. Cancer has become a major source of morbidity and mortality globally. In 2008, of the 12.7 million new cases and 7.6 million cancer-related deaths, 56% of these newly reported cases occurred in developing countries. Interleukin 6, a pleiotropic cytokine has both tumour-promoting and tumour inhibitory effects, It plays an important role in cancer pathogenesis

Aims and Objectives: The study aimed to evaluate the serum levels of interleukin 6 in breast cancer patients and normal controls. This is to access if an increase in the level of IL-6 is associated with the incidence of breast cancer.

Materials and Methods: The study included a total of ninety (90) subjects; sixty (60) breast cancer patients and thirty (30) normal controls. Serum interleukin 6 was analysed using sandwich ELISA obtained from Melsin Medical Corporation limited. CAT. NO: EKHU_0140. LOT #:M20180709. A p-value ≤ 0.05 was considered statistically significant.

Results: The findings of this study shows a significant increase in IL-6 with breast cancer patients (16.080 ± 0.71) compared to normal controls (10.310 ± 1.08) (P<0.05).

Conclusion and Recommendation: The result of this research has indicated a strong association between the level of IL-6 and breast cancer development but however, there is a need for a more robust further research study.

Keywords: Breast cancer, Interleukin-6, Chemotherapy, and Sandwich ELISA

Introduction

Breast cancer is a cancer that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, nipple discharge, or a red, scaly patch on the skin [1]. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin [2]

Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Cancers developing from the ducts are known as ductal carcinomas, while those developing from lobules are known as lobular carcinomas (WHO, 2014). Also, there are more than 18 other subtypes of breast cancer. Some cancers, such as ductal carcinoma in situ, develop from pre-invasive lesions [1]. Risk factors for developing breast cancer include being female, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, older age, and family history [3]. About 5–10% of cases are due to genes inherited from a person's parents, including BRCA1 and BRCA2 among others [1]. Breast cancer is the most common cancer occurring among women in Nigeria having overtaken cervical cancer [4]. The incidence rate of breast cancer among West African women are quite low compared to that of African American and Whites but have gradually risen in recent times from between 13.8 and 15.3 per 100,000 to 33.6 [5]. The incidence of breast cancer in Sokoto in 2011 was 7.4 per 100,000 women with 99% presenting with an advanced stage of the disease [6]. In the last few decades, there has been substantial progress in breast cancer management due to the advent of potent chemotherapy and guided therapeutic regimes. However, the prognosis of breast cancer is still considered as poor [7].

Cytokines are low molecular weight glycoproteins and they play an important role in cancer pathogenesis [8]. Each cytokine triggers certain cascades of events in their target cells by binding to their receptors and activating intracellular signal transduction pathway [8]. Interleukin-6 (IL-6) is a 23.7KD pro-inflammatory cytokine involved in acute phase response, differentiation of monocytes to macrophages, the proliferation of T cells and Th-2 cytokine production [9]. IL-6 as a major mediator of the inflammatory response plays a primary role in the pathophysiology of cancer [10]. Cancer cells exposed to IL-6 or which secretes the cytokine in an autocrine factor shows malignant features such as the enhanced capacity to invade the extracellular matrix and increased drug resistance [11]. Based on these data, the inhibitors of IL-6 or IL-6 receptors with specific

antibodies has been proposed as a support cancer therapy [12]. A wide repertoire of cytokines are secreted by the breast tumours with IL-6 being part of them [13]. The involvement of IL-6 at the cellular level is reflected by the results of serum studies of cancer patients, where IL-6 has been proven to be a valuable indicator of prognosis and tumour load [14]. Therefore, evaluating the serum level of IL-6 will prove beneficial in assessing the efficacy of chemotherapy. Cytokines and its implications in breast cancer is an evolving area of active research to help improve prognostications and therapeutic guidance, there should be a continuous Search for newer molecular markers, such as checking their levels. So this study aims to evaluate the levels of IL-6 in breast cancer patients attending oncology unit, UDUTH, Sokoto

Materials and Methods
Study Area
The study was carried out at Department of Immunology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto (UDUS) and Radiotherapy and Oncology unit, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, North-Western Nigeria. The Hospital is a tertiary institution located within the Sokoto metropolis. Sokoto is the capital city of Sokoto State located in the extreme North-west of Nigeria. The hospital receives referrals mainly from the neighbouring states of Kebbi, Zamfara and Niger. Sokoto is, on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under 40ºC (104.0ºF) and the dryness makes the heat bearable. The rainy season is from June to October during which shower is a daily occurrence.

Study Design
This is a case-control study design to determine the levels IL-6 in breast cancer patients attending the Radiotherapy and Oncology unit of Usmanu Danfodiyo University Teaching Hospital (UDUTH), and normal controls.

Study Subjects
A total of 60 Patients with Breast Cancer admitted at Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto and 30 healthy controls, who passed the inclusion criteria were randomly recruited for this study, after consenting to participate in the research

Data Collection
A semi-structured interviewer questionnaire was administered to all consenting breast cancer patients to obtain information on their social-demographic and medical history.

Informed Consent and Ethical Approval
Informed consent for inclusion in the study was obtained from the patients and controls using a standard informed consent form. Approval for the study was obtained from the Ethics and Research Committee of Usmanu Danfodiyo University Teaching Hospital.

Sample Collection
Five ml (5ml) of whole blood was aseptically collected using Monovette® blood collection bottles from 60 Breast cancer cases

and 30 healthy control subjects. The collected blood samples were labelled and then allowed to clot. Using a cold centrifuge, the clotted blood was centrifuged at 3000 RPM for 10 minutes. The serum was transferred into sterile serum separation tubes and was stored immediately at -20 until used.

Laboratory Analysis
The serum levels of interleukin 6 were determined quantitatively using the sandwich ELISA technique. The analysis was done in strict adherence to the manufacturer's instructions.

Data Analysis
Data obtained was be entered into SPSS version 20 for analysis. The results were expressed as mean ± SD. Group comparison was made using one-way analysis of variance (ANOVA) and Chi-square test while Student's T-test was used for paired comparison. The P-value of ≤ 0.05 was used to determine the level of statistical significance. The results obtained was presented using Tables.

Presentation of Results
A total of ninety (90) blood samples were collected out which, sixty (60) were from histologically confirmed breast cancer patients, attending Usmanu Danfodiyo University teaching, Sokoto and thirty (30) were from apparently normal adult females with no history of any inflammatory disease(Table 1).

Table 1: Sociodemographic Factors among Breast Cancer Patients.

Characteristics	Frequency (N)	Percentage (%)
Age Group (years)		
20-29	3	5
30-39	15	25
40-49	33	55
50-59	9	15
Total	60	100
Marital Status		
Married	51	85
Single	6	10
Widowed	3	5
Total	60	100
Residence		
Urban	42	70
Rural	18	30
Total	60	100
Occupation		
Employed	13	21.6
Unemployed/Housewife	47	78.8
Total	60	100

Table 1 shows the sociodemographic characteristics of the patients. The age range of 40 - 49 years was found to have the highest frequency 33 (55%), followed by 30 - 39 years 15 (25%), 50 - 59 years 9 (15%) and 20-29 years has the least frequency 3 (4.2%). Occupational distribution showed that, those who are unemployed/ full housewife have the highest prevalence rate of 47 (78.4%), while the employed ones have 13 (21.6%). The marital status of the patients shows that married women have the

highest frequency 51 (85%), followed by single 6 (10%), single 6 (8.3%) and Widowed 3 (5%). Most of the patients resided in the Urban areas 42 (70%) and 18 (30%) in the rurals.

Table 2: Results for Serum IL-6 in Breast Cancer Patients and Normal Controls.

Subjects	N	Mean ± SME (pg/ml)
Patients	60	16.080 ± 0.71
Controls	30	10.310 ± 1.08

Table: 2, This table shows a significant increases in serum IL-6 in breast cancer patients (16.080 ± 0.71) compared to normal controls (10.310 ± 1.08) (P<0.05). *N=numbers of subjects, pg/ml=picogram per miles*

Table 3: Shows Comparism of serum IL-6 in breast cancer patients and normal controls.

Subjects	N	Mean ± SME (pg/ml)	T	p-value
Patients	60	16.080 ± 0.71	-3.611	0.000
Control	30	10.310 ± 1.08		

Table 3, Shows a significance value P value of 0.000, in the level of serum IL-6 between the normal subjects and the cancer patients. N=number of subjects, p=level of significance, pg/ml= pictogram per miles.

Table 4: Associations between Sociodemographic factors and IL-6 levels in Patients.

Sociodemographics Factors		Mean ± SEM	p-value	X^2
Age Range	20– 29	14.28 ± 1.5	0.316	1.498
	30 – 39	15.43 ± 2.2		
	40– 49	16.31 ± 1.4		
	50 – 59	15.07 ± 1.9		
Marital Status	Single	14.03 ± 1.8	0.173	3.758
	Married	16.45 ± 1.8		
	Widowed	15.45 ± 1.8		
Occupation	Employed	15.66 ± 1.9	0.183	4.764
	Unemployed	16.82 ± 1.5		
Residence	Urban	16.45 ± 1.8	0.316	5.283
	Rural	15.40 ± 1.5		

Table 4: The Associations between Sociodemographic factors and IL-6 levels in cancer patients shows no level of significance across all the sociodemographic factors. SEM= standard Error of Mean, X^2= Chi-square value, p = Statistically Significant (p< 0.05)

Discussion

Cytokines have been reported to play crucial roles in the immunomodulation and immunopathogenesis of cancers. They have been found to have both tumour progressions and inhibitory effects. Interleukin 6, a pleiotropic cytokine has been found to be closely involved in several biological processes, which includes regulation of cell growth, proliferation and apoptosis.

The result of this study shows upregulation in the serum levels of IL-6 in breast cancer patients (16.080 ± 0.71) compared to normal controls (10.310 ± 1.08) (Table 2). This is in agreement with the work of Wang and Yang [15], in the study conducted on a Chinese population, where they reported a upregulations in the level of IL-6 compared to the controls. This upregulation according to their research might be due to the fact, that IL-6 acts as an autocrine growth factor in the pathogenesis of malignancy and also due to their involvement in several intracellular signalling pathways; For instance, the binding of IL-6 to its receptor could activate the Janus family of kinases bound to the cytoplasmic domain of gp130, which could promote its nuclear transfer and transcriptional function [15].

Aberrant expression of IL-6 as observed in this study maybe responsible and contributory to angiogenesis and metastasis in breast cancer, thereby contributing to metastatic potential as it up-regulates pro-angiogenic molecules, including vascular endothelial cell growth factor (VEGF) [16].

This research has shown no significant relationship between the level of IL-6, with regards to age groups, residence, occupation and marital status, although a slight increase is seen in the age group 40-49, Married subjects, unemployed and those living in urban areas. However, this contradicts a study by Kiecolt-Glaser *et al.,* [17] which showed a significant increase among older age groups due to stress and age-related diseases.

The age range of 40 - 49 years was found to have the highest frequency 33 (55%), this tallies with the work of Yu *et al.,* [18], which concluded that the breast cancer cases were primarily diagnosed in the 40–45 and 45–50 year age groups (21.6% and 30.3%,) respectively. Occupational distribution showed that those who are unemployed/ full housewife have the highest 47 (78.4%), while the employed ones have 13 (21.6%). The marital status of the patients shows that married women have the highest frequency 51 (85%), followed by single 6 (10%), single 6 (8.3%) and Widowed 3 (5%). Most of the patients resided in the Urban areas 42 (70%) and 18 (30%) in the rural. The incidence of breast cancer is associated with biological, behavioural and social factors therefore, comprehensive measures should be taken to prevent the occurrence of breast cancer [18].

Conclusions and Recommendations

Based on these research findings, it could be concluded that IL-6 play an active part in breast cancer pathogenesis and thus could serve as a treatment options, it is therefore recommended that, IL-6 or its receptors should be considered a therapeutic target in the treatment of breast cancer. More researches are therefore recommended into the levels of IL-6 at different cancer stages, with a larger population sizes.

Conflict of Interest: There is no conflict of interest to declare.

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