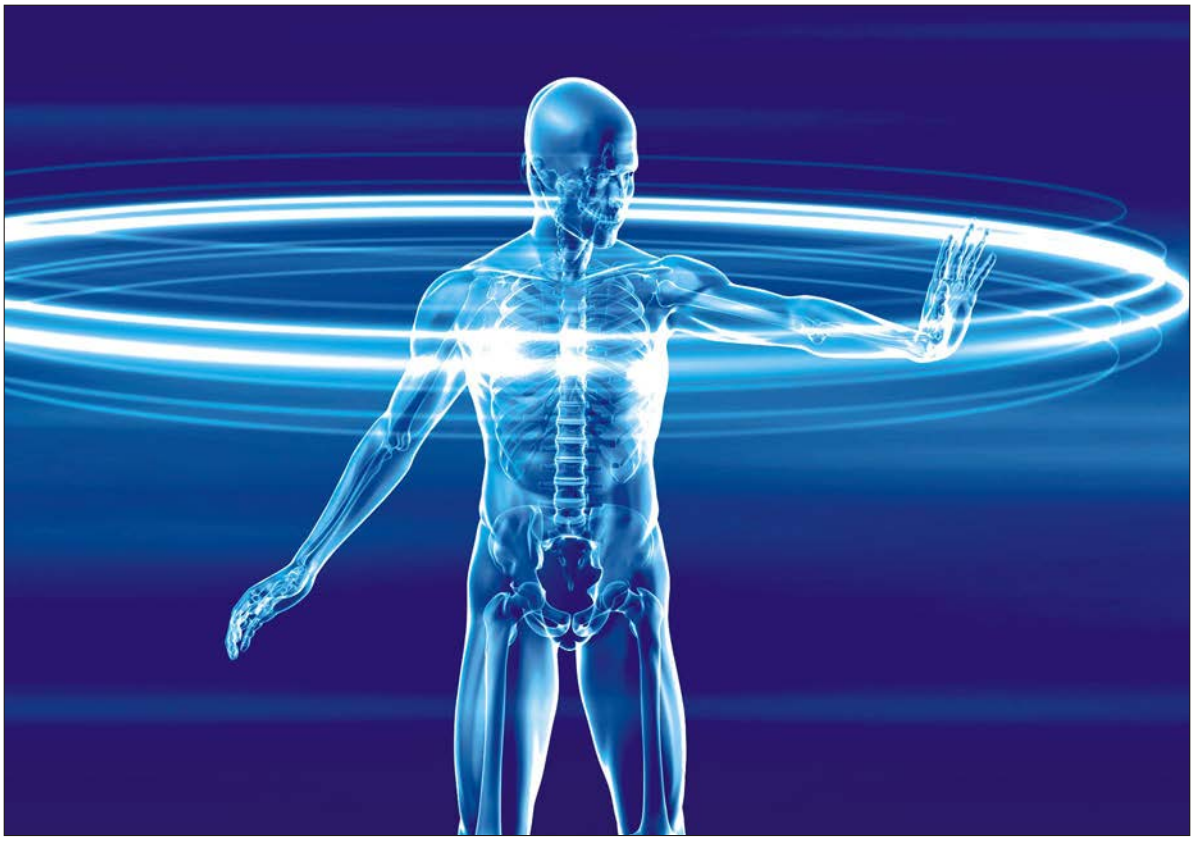

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Research Article
Breast Cancer in Nigerian Women: Evaluating the Utility of Circulating Immune Complexes and Cancer Antigens (CA 15-3 and CA 27.29) in Disease Surveillance

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Abstract

Background and Objective: Background breast cancer remains an important medical challenge, despite sustained global efforts at its prevention and control. Various factors are expressed in the serum during breast tumorigenesis and can be of value in the surveillance of the disease. Breast cancer can be affected by racial/ethnic, environmental and other variables. In our locality, there is scanty information on the value of these serum factors in screening and surveillance of breast cancer-hence the need for this study. **Methodology:** A total of 89 treatment naïve females (age 29-65 years), with clinically and pathologically confirmed breast tumour were recruited, along with apparently healthy control (N = 21) subjects. Patients with malignant tumour were further grouped into early stage and advanced stage. Serum from patients and controls were investigated for cancer antigens (CA 15-3, CA 27.29) and circulating immune complexes (CIC). The patients were subsequently subjected to standard treatment modalities. Follow-up investigations for the markers were carried out at intervals after treatment. Assays for serum the immunological factors were by immunoenzymatic methods. **Results:** Tumour of all forms recorded significantly higher pre-treatment mean values for CA 15-3 and CA 27.29, compared with the control group ($p<0.05$). Therapeutic interventions significantly reduced the values for CA 15-3 and CA 27.29 ($p<0.05$) but had no consistent effect on the level of CIC. The correlative significance between CIC and cancer antigens is weak. **Conclusion:** Tumour markers CA 15-3 and CA 27.29 showed promising diagnostic and prognostic potentials, while CIC appears to be of limited utility in the management of breast cancer in our environment.

Key words: Breast tumour, CIC, cancer antigens, treatment and disease progression

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Increasing reports suggest a world-wide increase in the incidence of breast cancer even among countries that previously have low prevalence such as African countries and African Americans in the US[1,2]. Breast cancer can be affected by racial/ethnic, environmental and other variables. African countries present with more aggressive, increase mortality, earlier age at presentation (35-45 years) and different pattern of gene expression[3].

The appearance of cancer and its progression pose great clinical unpredictability before and after diagnosis, therapy and appearance of recurrent secondary deposits and different biological markers have been used for diagnosis, prognosis and management of patients with cancer[4,5]. Human breast cancer cells have long been shown to possess tumour neoantigens[6]. Cancer antigen CA 15-3, a glycoprotein overexpressed in breast cancer and cancer antigen CA 29.27 are most widely tumour marker for breast cancer diagnosis[7] and useful in monitoring patients post-operatively for recurrence, particularly metastatic disease[8]. The immunogenicity of these antigens, the possible antibody response in cancer patients and the resultant interaction between specific antibodies and cancer antigens may result in the production of specific circulating immune complexes[9,10]. Circulating immune complexes have been detected in certain cancers of the mouth[11-13], esophagopharyngeal[14], lung[15], breast[16,17], colon and Burkett's lymphoma and their concentrations suggested to have possible relationship to the stages of the disease. A strong correlation is thought to exist between cancer antigens and circulating immune complex levels and progress to cancer and invariably in breast cancer.

Racial/ethnic variations in the expression of circulating immune complexes and other molecular profile proteins have also been documented[18-22]. The unique genetic features of racial groups in combination with environmental factors have been observed by many authors to influence carcinogenic mechanisms leading to biological differences in the molecular profile of a tumor[23,27]. Therefore, the interactions between environmental and genetic factors should be considered when determining cancer susceptibility, regardless of intrinsic genetic differences between groups. The importance of the immunocomplex CIC and their relation with several diseases have been documented[7,8,28]. All the studies however were in Caucasians and patients from Asian ancestries. There is need therefore for similar studies in black Negroid race of Africa aimed at establishing Standards, Options and Recommendation (SOR) peculiar to the race. In this study, attempts were made to establish possible correlative significance between the detection and concentration of cancer antigens (CA 15-3 and CA 27.29) and circulating immune complexes and disease progression in women with premalignant conditions of the breast and those with various stages of breast cancer before and after treatment.

MATERIALS AND METHODS

This is a prospective longitudinal study conducted between February, 2015 and June, 2017 on female patients referred to the Oncology Units of Departments of Surgery of University of Nigeria Teaching Hospital, Enugu and Federal Teaching Hospital, Abakaliki (both in Eastern-Nigeria). Diagnosis and staging were clinically and pathologically confirmed. Studied individuals were grouped into three:

Group I: Included 68 breast cancer patients, due to small sample size the patients were further divided into early stage (stages 1 and 2, N = 28) and advanced stage (stages 3 and 4, N = 40)

Group II: Included individuals with benign breast tumour (N = 21)

Group III: Apparently healthy age/sex-matched control (N = 21)

Sampling was by self-selection following the approval of the study protocol by the respective Hospitals Ethical Committee, informed written consent obtained from the individuals and exclusion criteria applied. Breast tumour patients who received any therapy prior to diagnosis (surgery/radiotherapy/chemotherapy), previous history of malignancy and history of any other medical illness, which would otherwise limit the survival of the patient in the absence of malignancy, were excluded. All patients underwent standard treatment modalities (neoadjuvant or adjuvant chemotherapy, radiotherapy, chemoradiation and/or surgery, depending on the stage of presentation. In benign and malignant tumor patients, blood samples were collected before any form of treatment and two more samples at 3 and 6 months interval. Apparently healthy sex/age-matched controls were recruited from the hospitals and university staff. The samples were centrifuged and serum separated and stored at -20°C until analyzed.

Analysis: The cancer antigens (CA 15-3 and CA 27.29) and CIC were measures by quantitative ELISA technique (kits produced by Diagnostic Automation, Inc. Calabasa, CA91302, cat #6333Z and CUSABIOR, cat #CSB-E13858h for cancer

antigens respectively and abcamR, ab178665 for CIC. All tests were done in duplicate and average results recorded. Data were analyzed using statistical package for Social Sciences (SPSS) software. Statistical significance was set at $p<0.05$. Dunn's multiple comparison tests and analysis of variance (ANOVA) were applied to evaluate differences in values among groups. GraphPad prism version 6.0 (by GraphPad, USA) was used for the graphs.

RESULTS

Overall, about 30% of both the breast cancer and benign breast tumour patients presented with CIC activity greater than 30 μg mL^{-1} CIC-C3d concentration. There were variations in the mean values of CIC across disease and treatment groups but the CIC level only differed significantly ($p = 0.0058$) between 3 months post-treatment in early stage breast cancer and pre-treatment benign breast tumour levels (Table 1, Fig. 1). Only a few (0.5%) of apparently healthy age/sex matched individuals presented with CIC activity above normal.

The majority, 94% of the breast cancer patients had pre-treatment CA 15-3 values above the recommended normal healthy range of below 35 U mL^{-1}. The mean values and statistical comparison of cancer antigens are presented in Table 2 and Fig. 2. The cancer antigens (CA 15-3 and CA 27.29) differ significantly ($p<0.05$) in mean serum levels across disease and treatment groups, respectively (Table 2 and 3, Fig. 2 and 3). The 6 month post treatment CA 15-3 level was significantly lower in the early stage breast cancer (ESBC) compared to other groups including diagnostic levels ($p<0.0001$). The CA 27.29 level was significantly lowest in the 6th month post treatment level and the median CA 27.29 level in 6 month post treatment returned to levels similar to that of apparently healthy control group.

Table 4 shows the relationship between CIC and CA 15-3 across the disease and treatment groups. In early stage breast cancer there was negative relationship ($r = 0.646$, $p<0.05$) at pre-treatment value, however the relationship tends positive at 3 months and 6 months. In advanced stage breast cancer there were negative relationships in the three treatment groups.

Similar relationships were observed between CIC and CA 27.29 across the disease and treatment groups (Table 5). The benign breast tumour group showed significant positive relationships at the treatment stages.

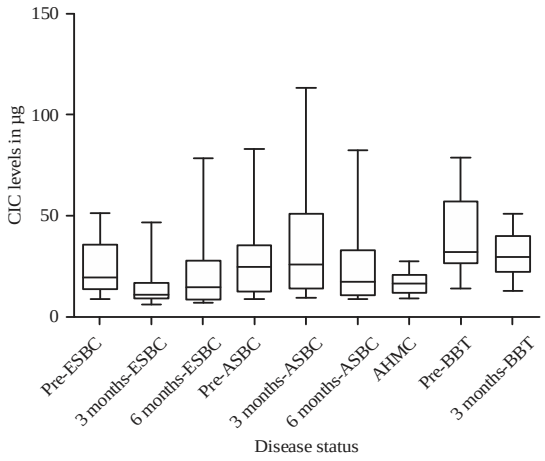

Fig. 1: Comparison of CIC levels across disease and treatment groups

ESBC: Early stage breast cancer, ASBC: Advanced stage breast cancer, AHMC: Apparently healthy-matched control, BBT: Benign breast tumour

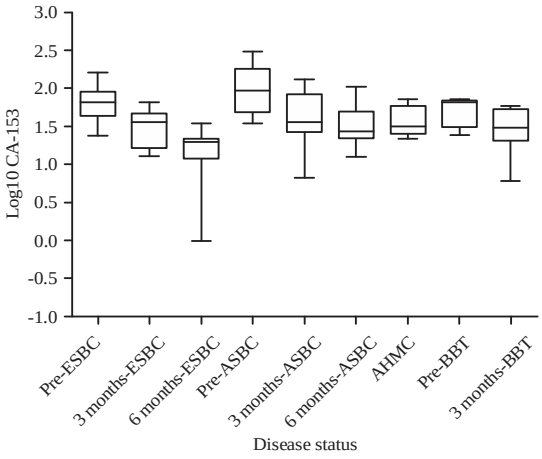

Fig. 2: Comparison of CA 15-3 levels across the disease and treatment groups

ESBC: Early stage breast cancer, ASBC: Advanced stage breast cancer, AHMC: Apparently healthy control, BBT: Benign breast tumour

Table 1: Summaries of CIC levels (μg mL^{-1}) across disease and treatment groups

CIC μg mL	Pre-ESBC	3 months -ESBC	6 months -ESBC	Pre-ASBC	3 months -ASBC	6 months -ASBC	AHMC	Pre-BBT	3 months -BBT
Mean	23.98	15.73	21.23	27.81	34.53	27.31	16.82	41.24	30.38
Standard deviation	14.93	12.35	20.74	21.27	27.29	24.03	5.831	21.82	12.47
Standard error of mean	4.310	3.565	6.254	4.755	6.103	6.421	1.844	6.901	4.156
Lower 95% CI of mean	14.49	7.879	7.292	17.85	21.75	13.43	12.65	25.63	20.79
Upper 95% CI of mean	33.46	23.57	35.16	37.76	47.30	41.18	20.99	56.85	39.96

ESBC: Early stage breast cancer, ASBC: Advanced stage breast cancer, AHMC: Apparently healthy control, BBT: Benign breast tumour

Table 2: Summary of statistics for CA 15-3 across disease and treatment groups

CA 15-3 U mL^{-1}	Pre-ESBC	3 months -ESBC	6 months -ESBC	Pre-ASBC	3 months -ASBC	6 months -ASBC	AHMC	Pre-BBT	3 months -BBT
Minimum	24	13	1	35	6.8	13	22	25	6
25%	45	16	12	50	27	22	26	32	21
Median	67	37	20	95	37	28	32	67	31
75%	93	48	22	186	84	50	60	70	54
Maximum	162	66	35	311	134	107	74	74	60
Mean	74	35	17	128	53	38	40	54	35
Standard deviation	38.0	17.7	9.8	82.5	37.0	26.1	19.9	21.0	18.8
SE	11.0	5.1	2.9	18.4	8.3	7.0	6.3	6.6	6.3
Lower CI of mean	50	24	11	89	36	23	26	39	21
Upper CI of mean	98	46	24	166	70	54	54	69	49

ESBC: Early stage breast cancer, ASBC: Advanced stage breast cancer, AHMC: Apparently healthy control, BBT: Benign breast tumour

Table 3: Summary of Statistics for CA 27.29 levels across disease and treatment groups

CA 27.29 U mL^{-1}	Pre-ESBC	3 months -ESBC	6 months -ESBC	Pre-ASBC	3 months -ASBC	6 months -ASBC	AHMC	Pre-BBT	3 months -BBT
Minimum	2.1	3.8	0.2	4.5	0.1	0	1.8	5.9	5.1
25%	6.1	5.45	2.3	5.625	2.375	4.6	2.5	6.125	5.9
Median	16.1	10.55	4.8	11.9	5.25	8.4	3.15	7.95	6.3
75%	21.88	15.58	5.9	24.93	7.175	10.5	5.6	10.43	8.9
Maximum	45.3	57.1	8.8	156.3	46.1	44.1	6.2	25.8	10.1
Mean	17.16	13.76	4.618	29.3	7.235	10.45	3.76	9.79	7.12
Standard deviation	13.53	14.48	2.689	46.33	9.807	11.44	1.594	6.057	1.73
SE	3.905	4.181	0.8108	10.36	2.193	2.955	0.504	1.915	0.5773
Lower CI of mean	8.564	4.557	2.812	7.616	2.645	4.116	2.62	5.457	5.79
Upper CI of mean	25.75	22.96	6.425	50.98	11.82	16.79	4.9	14.12	8.45

ESBC: Early stage breast cancer, ASBC: Advanced stage breast cancer, AHMC: Apparently healthy-matched control, BBT: Benign breast tumour

Table 4: Relationship between CIC and CA 15-3 across disease and treatment group

Stages	Parameters	r	Equation	p-value
ESBC	CIC pre	0.646	= 43.782-0.237 (CA 15-3)	0.000*
	CIC 3 months	0.259	= 9.329+0.206 (CA 15-3)	0.182
	CIC 6 months	0.522	= 2.236+0.995 (CA 15-3)	0.006
ASBC	CIC pre	0.196	= 34.230-0.050 (CA 15-3)	0.226
	CIC 3 months	0.262	= 44.765-0.193 (CA 15-3)	0.102
	CIC 6 months	0.024	= 25.045-0.018 (CA 15-3)	0.886
AHMC	CIC pre	0.325	= 20.028-0.077 (CA 15-3)	0.220
	CIC 3 months	0.325	= 20.028-0.077 (CA 15-3)	0.220
	CIC 6 months	0.325	= 20.028-0.077 (CA 15-3)	0.220
BBT	CIC pre	0.042	= 41.172-0.043 (CA 15-3)	0.849
	CIC 3 months	0.055	= 30.373-0.036 (CA 15-3)	0.807
	CIC 6 months	-	-	-

ESBC: Early stage breast cancer, ASBC: Advanced stage breast cancer, AHMC: Apparently healthy-matched control, BBT: Benign breast tumour

Table 5: Relationship between CIC and CA 27.29 across disease and treatment group

Stages	Parameters	r	Equation	p-value
ESBC	CIC pre	0.077	= 26.250-0.109 (CA 27.29)	0.698
	CIC 3 months	0.468	= 11.055+0.400 (CA 27.29)	0.012
	CIC 6 months	0.361	= 8.082+2.752 (CA 27.29)	0.070
ASBC	CIC pre	0.090	= 29.016-0.042 (CA 27.29)	0.579
	CIC 3 months	0.345	= 41.479-0.961 (CA 27.29)	0.029
	CIC 6 months	0.092	= 26.301-0.196 (CA 27.29)	0.583
AHMC	CIC pre	0.288	= 20.357-0.965 (CA 27.29)	0.280
	CIC 3 months	0.288	= 20.357-0.965 (CA 27.29)	0.280
	CIC 6 months	0.288	= 20.357-0.965 (CA 27.29)	0.280
BBT	CIC pre	0.417	= 24.225+1.554 (CA 27.29)	0.048
	CIC 3 months	0.470	= 5.743+3.312 (CA 27.29)	0.027
	CIC 6 months	-	-	-

ESBC: Early stage breast cancer, ASBC: Advanced stage breast cancer, AHMC: Apparently healthy-matched control, BBT: Benign breast tumour

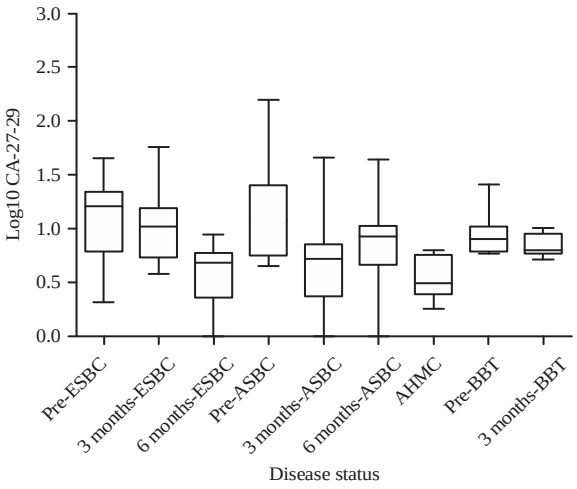

Fig. 3: Comparison of CA 27.29 levels across the disease and treatment groups

Key: ESBC: Early stage breast cancer, ASBC: Advanced stage breast cancer, AHMC: Apparently healthy-matched control, BBT: Benign breast tumour

DISCUSSION

Human cancer cells have long been shown to possess tumour neoantigens[6]. The immunogenicity of cancer antigens, the possible immune response in cancer patients and the resultant interaction between specific antibodies and cancer antigens may result in the production of specific circulating immune complexes[9,10].

Circulating immune complexes have been detected in certain cancers of the mouth[11,12], esophagopharyngeal cancer[14], lung cancer[15], breast cancer[16], colon cancer[29], Burkitts lymphoma, their concentrations suggested to have possible relationship to stages of the disease and when deposited on tissues play a specific role as initiators of mechanism of tissue injury.

In this study an immunoenzymatic assay for the quantitative measurement of CIC-C3d in human, that has been found reliable[13] was adopted and used to assay CIC levels in patients with breast tumour before and at intervals after any form of treatment. We observed variations in mean CIC levels between disease and treatment groups but the differences were not significant. This is similar to the findings in oral leukoplakia patients[13] and lung cancer[15]. However significant difference (p value 0.0058) was observed between 3 months in early stage breast cancer (ESBC) and pre-treatment benign breast tumour (BBT). The reason for this isolated case could not be explained probably due to individual immunological variations. This finding is in variance with previous works in breast cancer[16,30] using radioimmunoprecipitation methods. The researchers observed that after mastectomy patients identified clinicopathologically as having a good prognosis had almost normal levels of immune complexes. The clinical utility and monitoring of breast cancer by circulating immune complexes was collaborated by other researchers[31-33]. All these studies nevertheless were done in western countries with different patterns in gene expression as well general health and environmental factors. The environmental, ethnic/racial implications of this immunological factor should always be considered in clinical applications.

The present study however, could not associate CIC to the disease burden since there were no significant variations across the disease and treatment groups. Although some patients with breast cancer as well as patients with benign breast tumour presented with elevated CIC activities, the results did not differ significantly in course of treatment, hence the diagnostic utility is queried. This could possibly be due to increased triggers for immunocomplex formation in this environment-infections (especially parasitic and bacteria associated with unhealthy environment) and other inflammatory conditions. We could not possibly exclude all the triggers for the elevation of CIC even in the apparently healthy control groups. For CIC to be of good clinical and diagnostic value there should be marked initial elevation that drops as the treatment of the disease is initiated.

Since, the CIC estimation is not antigen specific, insignificant or no variation in course of treatment may be due to other antigens and not the specific antigen. For CIC estimation to be of clinical utility in this environment with multiple factors associated with immunocomplexes formation, there is need for the development of improved and antigen specific CIC methodology for the estimation of specific immune complexes.

Clinical evidences of the association of cancer antigens, CA 15-3 and CA 27.29 among others have been documented[7,8,34] and shown to be directly related to tumour burden and may be independent prognostic factors for breast cancer. Cancer antigen CA 15-3 concentration has been used to monitor the course of disease and therapy in metastatic breast cancer patients. The CA 27.29, also called breast carcinoma-associated antigen, is used as a marker for breast cancer[7], check for recurrences of cancer in previously treated women and help to monitor treatment response and identify recurrence.

Our findings showed statistical significant differences in CA 15-3 and CA 27.29 levels across disease and treatment groups. These markers therefore may be used for diagnosis

and most importantly for prognosis. The fact that about 60 and 30% of patients with benign breast tumour and apparently healthy control groups respectively presented with increased concentration of pre-treatment CA 15-3 gave mixed feelings on the diagnostic utility of this marker. However, once the base line elevation in breast cancer has been established, CA 15-3 may be used for prognosis and to check for recurrences of cancer in previously treated women.

Similar results on the clinical utility of breast cancer antigen (CA 15-3 and CA 27.29) were reported[35-41] affirming that they could be used to monitor response to breast cancer treatment and disease recurrence. The elevation in CA 15-3 serum level (>25-40 U mL^{-1}) usually correlates with tumor malignancy.

It is important to note that other cancers (colon, liver, lung, pancreatic, ovarian and prostate), can produce CA 15-3 and CA 27.29[7,8,34] but some non-life-threatening conditions may also cause these antigens to show up in the blood, as in ovarian cyst and benign conditions of the breast, liver and kidneys. Cancer antigen 27.29 is used to monitor metastatic carcinoma of the breast. The estimations are useful when monitoring both the course of disease and response to therapy since there is a direct correlation between the changing levels and clinical status[36]. In women with known metastasis, reductions in levels of these markers indicate good response to treatment while increasing levels indicate resistance to therapy and progressive disease.

In this study, decreasing patterns in mean values for CA 15-3 and CA 27.29 were observed at 3 and 6 months after any form of treatment, confirming a statistical significant differences across disease and treatment groups. Although only the minority (16%) of the breast cancer patients presented with increased concentration (above normal) of CA 27.29, the absence of positivity in the benign breast tumour and apparently healthy control groups possibly suggest better and more specific diagnostic utility of CA 27.29 in breast cancer.

The CA 27.29 level was significantly lowest in the 6th month post treatment level (p value 0.001). Interestingly the median CA-27-29 level in 6 month post treatment returned to levels similar to that of apparently healthy control (AHMC) group. This indicates that Cancer antigen 27.29 could be used to monitor response to breast cancer treatment and disease recurrences. The result is in parity with other findings[42,43] further confirming that elevated serum CA 27.29 levels found in women in remission for breast cancer indicated a significant possibility for cancer reoccurrence. The authors opined that although these tests, (CA 15-3 and CA 27.29) may not be used as diagnostic screening tests, the knowledge about these cancer biomarkers however, may provide great opportunities for improving the management of cancer patients by enhancing the efficiency of detection and efficacy of treatment.

The varied and weak relationship between the CIC and cancer antigens (CA15-3 and CA 27.29) could be due to the fact that serum immunocomplex formation is not only specific to the cancer antigens and the procedure for CIC measurement is not specific. There may be other antigens involved in the immunocomplex formation other than studied cancer antigens. There is need therefore for the development of antigen specific CIC estimation procedure and search for possible new cancer antigens in our environment.

Research for other immunological factors and tumour markers that may be of diagnostic and prognostic utility in the management of breast cancer and cancers in general is advocated.

CONCLUSION

Tumour markers CA 15-3 and CA 27.29 showed promising diagnostic and prognostic potentials and could be used in surveillance and management of breast cancer while CIC appears to be of limited utility in the management of breast cancer in our environment.

SIGNIFICANCE STATEMENT

The study discovered that the CA 15-3 and CA 27.29 tumor marker could be used for the diagnosis of breast cancer and also to manage it hence with lowered utility. This study would help researchers to assess these biomarkers in detail and manage the tumor. Thus the best theory on it may be arrived at.

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