Evaluation of C-Peptide in Breast Cancer Patients in Anambra State, Nigeria

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ABSTRACT

Breast cancer is the most common malignancy among women worldwide, and despite advances in detection and treatment, there is an ongoing need for reliable biomarkers that can aid in early diagnosis, prognosis, and monitoring of treatment response. C-peptide have been proposed as potential biomarkers in breast cancer. There is a scarce information on the relationship between C-peptide with cancer progression and prognosis and none of this information are related to Nigeria or any race in the country, Nigeria. This study aims to evaluate the levels of C-peptide in patients with breast cancer. The specific objectives were to assess and correlate the results obtained from C-peptide among breast cancer patients and healthy volunteer control individuals and their anthropometric measurement in Nnewi-North, Anambra state. Approvals for ethical consideration were obtained from the Ethical Committee of Ministry of Health, Anambra State of Nigeria. The study was carefully explained to the subjects and their informed consent obtained before they were recruited into the study. Including: Forty-five (45) breast cancer patients and forty-five (45) apparently healthy control individuals. The samples in the plain sample containers were spun for 10 minutes at 3000 revolutions per minute (RPM) and separated. Serum C-peptide was estimated using the sandwich ELISA Method. The outcomes of this study were statistically evaluated using SPSS software version 23.0, which is a statistical tool for social sciences. The result revealed that no significant difference was observed in the level of C-peptide (1.40±1.67 ng/mL) of the women with breast cancer from that of the control group (1.54±1.36 ng/mL) and (0.58±1.56ng/mL) respectively. No significant difference ($p>0.05$) in the serum C-peptide level between the age group. No significant correlation ($p>0.05$) between C-peptide level in the test and the control group and the anthropometric parameter. This study recommends that further research should be carried out to determine whether C-peptide have subtype-specific prognostic value in breast cancer and to explore other metabolic pathways that may influence disease progression.

INTRODUCTION

Background of Study

Cancer is a major health problem worldwide and is the second leading cause of death in the United States (Rebecca *et al*., 2023). Cancer is one of the diseases which causes high death rates in these recent times and has resulted in the increased rates of death yearly (Chhikara and Parang, 2022).

Cancer is a disease in which there's pathophysiological alterations or mutation in the inherent process of cell division. This has emerged as a significant disorder responsible for a large number of death year by year worldwide. More than 19.3 million (19,300,000) new cancer cases were diagnosed and reported recently, leading to approximately 10 million deaths in 2020 based on the reported data (Chhikara and Parang, 2022). The continuous emerging incidences of cancer worldwide that causes millions of deaths annually have generated the need and demand for developing potent pharmaceuticals for treating different cancers. The cancer generation can be due to many factors such as internal stress, heredity as well as environmental factors. The responsible factor varies from patient to patient and it depends on the type of cancer and it's localisation. The treatment needs to be established adequately in each respective case (Chhikara and Parang, 2022). Environmental and climatic change due to industrialization as well as lifestyle and food style, is considered one primary concern for

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increasing numbers of cancer incidences. However, a proper rational link stills needs to be validated to establish any valid conclusive claim. The affected organs of origin identifies the type of cancer . Increased prevalence in different gender or population has been observed with occurrence of a particular kind of cancer. For example, breast cancer is most prevalent type of cancer in women worldwide, while lung and prostate cancer are the primary incidence in male population. lung cancer is the second major cancer in males and females when counted combined (Chhikara and Parang, 2022).

Breast cancer, the most frequent disease in women worldwide, represents a significant public health concern on a global scale. Breast cancer is a set of physiological and molecular heterogeneous diseases that begin in the breast. Advanced breast cancer with distant organ metastases is considered incurable with current therapies. Aside from new treatment improvements, the next global challenge in breast cancer care is equal worldwide access to therapeutic advances (Ali, 2023). The incidence of breast cancer continues to rise in every part of the world. Despite advances in its identification and treatment, which have resulted in lower mortality rates, it appears vital to seek out new therapeutic approaches, predictive and prognostic indicators. The article provides a literature review of breast carcinoma, a condition that affects women worldwide. As a result, women should be aware of the disease's course, the importance of regular screenings for early detection of breast cancer, and the best treatment options (Ali, 2023).

Female breast cancer has overtaken lung cancer as the most commonly diagnosed cancer worldwide. The estimated new breast cancer cases reached 2.3 million in 2020, accounting for 11.7% of all new cancers,and 684,996 cases died of it. In China, breast cancer was the most common malignancy among women, with an estimated number of 306,000 new cases occurring in 2016. The incidence of breast cancer has increased since the widespread uptake of mammography screening and continues to increase with the aging of the population. Globally, death rates for female breast cancer were conspicuously higher in transitioning countries (15.0 per 100,000) versus transitioned country (12.8 per 100,000) (Hong, 2022).

C-peptide (connecting peptide) is a polypeptide consisting of 31 aminoacids residue and a molecular weight of 3020.29 g/mol. It carries a negative charge and lacks a tertiary structure under physiological conditions. The isoelectric point (pl) of c-peptide is 3.45 due to the large number of negatively charged aminoacids. The peptide does not contain residues of aromatic aminoacids and demonstrates good solubility in aqueous Solvent (Poteryaeva and Usynin, 2021). C-peptide enters the blood at a concentration equimolar to insulin. The half-life of insulin in peripheral blood is about 4mins.

The main part of insulin (80%) undergoes proteolytic degradation in the liver, the rest degrades in the kidneys, fat and muscle cells. C-peptide is not removed from the blood stream by the liver; it's half-life is about 30mins in healthy people and even longer in diabetic patients. C-peptide is catabolized mainly in the kidneys, with only a small portion being excreted in the urine. Therefore, by the content of c-peptide in the blood, the insulin-secreting ability of the islet apparatus of the pancreas can be easily examined (Poteryaeva and Usynin, 2021). C-peptide is a marker of pancreatic insulin secretion. Recent studies indicate that elevated C-peptide levels are associated with an increased risk of breast cancer. A population-based case-control study found that women in the highest tertile of C-peptide concentration had an odds ratio for breast cancer compared to those in the lowest tertile, independent of traditional risk factors such as body mass index (BMI) and menopausal status (Zhang *et al.,* 2021).

Another study highlighted that high C-peptide levels were linked to a 39% increased risk of breast cancer, particularly in women aged 51 and older (González *et al.,* 2021). These findings support the hypothesis that C-peptide may play a significant role in breast cancer development through insulin resistance mechanisms.

Specific studies focusing on C-peptide levels in Nigerian breast cancer patients are scarce. However, research has been conducted on other serum biomarkers.

Given the global findings, investigating C-peptide levels in Nigerian breast cancer patients could provide valuable insights into their potential roles as biomarkers in this population. This research is warranted to explore these associations and their implications for breast cancer diagnosis and management in Nigeria.

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Statement of Problem

Breast cancer is one of the most common malignancies affecting women worldwide, leading to significant morbidity and mortality. Despite advances in screening, diagnosis, and treatment, the variability in disease outcomes and the challenge of predicting prognosis remain critical issues. The identification of reliable biomarkers that can aid in early detection, assess prognosis, and guide treatment strategies is essential to improving patient outcomes. C-peptide, a byproduct of insulin production, a glycoprotein often associated with liver cancers, have been suggested as potential biomarkers in various cancers. However, their roles in breast cancer, particularly in terms of their correlation with disease progression, prognosis, and response to treatment, are not well understood.

The problem addressed by this study is the lack of comprehensive data regarding the association between C-peptide levels in breast cancer patients. This gap in knowledge limits the ability of clinicians to use these markers effectively in routine practice, potentially missing opportunities for improved personalized care. Therefore, this study aims to assess the levels of C-peptide in patients with breast cancer and investigate their potential as biomarkers for disease progression and prognosis, with the goal of contributing to more precise and effective management strategies for breast cancer patients.

Justification of Study

Breast cancer is a leading cause of cancer-related deaths among women globally, with increasing incidence rates in many parts of the world. Preliminary studies suggest that C-peptide might be linked to breast cancer. This study is justified by the need to fill the existing gaps in the understanding of the roles of C-peptide in breast cancer. By investigating these biomarkers, the study aims to provide new insights that could lead to more effective and tailored management strategies for breast cancer patients, potentially improving survival rates and quality of life. This then remains the focus of the work.

Additionally, this research could pave the way for this study to explore the mechanistic links between these biomarkers and breast cancer, ultimately contributing to the broader field of cancer research and biomarker discovery.

This research is also carried out to determine the prevalence of high C-peptide in breast cancer patients in Anambra state, as well as estimate if age predisposes prevalence of the disease.

Aim of Study

To assess the roles and level of C-peptide in patients with breast cancer in Nnewi-North, Anambra state, Nigeria.

Specific Objectives

1. To assess and estimate the results obtained from C-peptide among breast cancer patients and healthy volunteer control individuals.
2. To assess and estimate the values of C-peptide obtained among different age groups in the study area.
3. To assess if there is any correlation in the level of C-peptide and their anthropometric measurement.
4. To assess if high C-peptide are prevalent among breast cancer patients in Nnewi-North, Anambra state.

Scope of Study

This study compared the levels of serum C-peptide in breast cancer patients and healthy volunteer control individuals (apparently control individuals that were screened).

The study will cover breast cancer patients at Nnewi-North, Anambra state, who are in or out patients of the participating hospital wards with breast cancer but excluded other patients who comes to the hospital for other reasons.

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MATERIALS AND METHODS

Area of study

The study was carried out in Anambra state, Nigeria. Subjects was recruited from NAUTH, Nnewi, Anambra State, Nigeria. Anambra State is a Nigerian Igbo speaking state located in the South-eastern region of the country. The state was created on 27 August 1991. Anambra state is bounded by Delta State to the west, Imo State and Rivers State to the south, Enugu State to the east and Kogi State to the north. The state's capital is Awka, while the state's largest city is Onitsha which is regarded as one of the largest metropolis in Africa. Nnewi is the second largest commercial and industrial city in Anambra State, and also a known automobile hub within Nigeria and Africa.

Subject

The group of patients was recruited from Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State. Between September 2024 to 1st week of February 2025, a cross-sectional study of fifty (45) Breast Cancer patients, females aged 18 to 60 years and forty (45) apparently healthy volunteer control subjects, females aged 18 to 60 years, that served as the group of which was recruited from anywhere in Anambra state.

Study Design

This research used a cross-sectional study design, which is suitable for assessing the prevalence of specific characteristics in a defined population at a single point in time. The cross-sectional design allows the simultaneous evaluation of C-peptide levels in Anambra State, facilitating a comprehensive understanding of their interrelationships in breast cancer patients.

Sample Size

Sample size was calculated using G*Power software (version 3.0.10). Power analysis for two independent groups was conducted in G*Power to determine a sufficient sample size using an alpha of 0.05, a power of 0.80 and a medium effect size (d=0.50). Based on these assumptions, the calculated total sample size of 90 (45 per group) has 80% power to detect a difference of 0.50 (medium effect size) at significance level of 0.05.

Study Population

The target population for this study consist of female patients with breast cancer within the age of 18 years to 60 years in Anambra State**.**

Inclusion Criteria

The study inclusion criteria include female participants, already diagnosed patients living with breast cancer and the control individuals with the age bracket (18 to 60 years), who gave their consent were incorporated.

Exclusion Criteria

The study exclusion criteria include female participants with HIV and control group outside the age bracket will be excluded. Also, the participants with additional conditions that could affect the results such as pregnant women/lactating mothers, liver failure, kidney failure, tuberculosis, heart failure history and history of harmful alcohol or substance use were excluded.

Informed Consent

Informed consent of the participants was obtained before sample collection. A convenient sampling technique was used to enroll participants. The selection was done based on those who will willingly give their consent to participate in the study after discussing the study design with them.

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Ethical approval

The ethical approval for this research was obtained from the Ethics Committee of Ministry of Health, Anambra State of Nigeria with the reference number MH/COMM/523/VOL.83.

Data Collection

Data collection will occur through a combination of laboratory tests and patient interviews.

Anthropometric Measurements

Weight Measurements: The participants' weight was evaluated with a bathroom scale (Gulfex Medical and Scientific, England). The weight was measured in kilograms (kg) and recorded to the nearest 0.1kg. After nulling the scale to zero, each measurement was taken. Participants were measured while standing barefooted, wearing light clothing with pockets empty, headgear and excessive hair accessories removed, and their arms swinging naturally by their sides.

Height Measurements: With the buttocks, upper back, or head touching the measuring surface of the rule, the participant's height was measured in meters using a height scale calibrated in centimeters (stadiometer), and the reading was taken to the nearest 0.1cm value.

Body Mass Index (BMI): Each adult participant's BMI was calculated by dividing their weight in kilograms by the square of their height in meters (kg/m2) as a measure of generalized obesity.

Blood Pressure Measurement:

Participants Preparation

All apparel that concealed the cuff placement position was removed from the participants. The participants was sitting comfortably, with their legs uncrossed and their backs and arms supported so that the center of the upper arm cuff was at the level of the right atrium (the midpoint of the sternum). They were told to stay as relaxed as possible and not to speak throughout the measurement.

Measurement: Blood pressure (BP), systolic, and diastolic pressure readings was taken from the participant's left arm using a sphygmomanometer (Omron Medical, United Kingdom) after being seated for ten minutes. The reading was taken in the morning to the nearest mmHg. Each participant was allowed to rest in this position for 10 minutes before the blood pressure was taken.

The cuff was tied around the participant's left arm, with the participant sitting comfortably on a chair with back support. The brachial artery was occluded and gradually deflated, and knockoff sounds detected(stethoscope) held over the artery. This sound was monitored attentively until it completely disappeared. The mercury level in the sphygmomanometer scale where the first clear tapping sound appeared corresponded to the systolic blood pressure. The mercury level in the sphygmomanometer where the sound disappeared corresponded to the diastolic blood pressure.

The readings will be taken in both arms, and the average will be recorded as the participant's blood pressure. Two readings will be taken for each participant each time, and the average of the two readings computed and used as the participants' blood pressure for the study.

Questionnaire

The collection of samples from breast cancer patients who are in or out patients of participating hospital, those who have provided informed consent was eligibly enrolled. A one-on-one discussion was carried out with the two groups. Their epidermiolocal data(age), sociodemographic variables and relevant history was collected using standardized questionnaire. Those who expressed interest in the study was given a questionnaire to complete. Those who met the eligibility requirements was enrolled in the study.

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Sample Collection

Venous blood sample of five (5) mL was collected from each individual by venous puncture using disposable syringe and the blood was dispensed into a plain container and allowed to clot and retract then it was centrifuged at 4000rpm for 10 minutes then the serum was extracted and dispensed into another plain container which was accurately labelled with a code specific to the individual and the samples was transported to the laboratory and stored at -20℃ until testing.

Determination of serum C-peptide

The level of C-peptide in serum was determined using Sandwich ELISA Method

Principle of sandwich ELISA

Sandwich ELISA works on the principle of antigen-antibody reaction where the target antigen is detected via anchoring between two antibodies, which recognize different epitopes. Sandwich ELISA starts from the immobilization of an antibody, called a capture antibody, on the microtiter plate.

After blocking the plate surface to avoid non-specific adsorption of other proteins, the antigen in the sample is allowed to react with the immobilized capture antibody, and the antigen bound to the capture antibody is then sandwiched with an enzyme-labeled antibody for color development which is then read spectrophotometrically

Reference Range: 0.56 - 3.25 ng/ml

Procedure of the Test

All reagents were brought (microplate wells) to room temperature (18 - 25℃) before use. The concentrated solution was diluted i.e 1 volume of wash buffer concentration with 49 volume of distilled water. Pipetted standard of 50ml, serum samples and control was put into the desired number of coated wells, fixed on the holder.

Enzyme's conjugate (100ml) was added into the same coated wells. It was mixed efficiently and incubated at room temperature for 60 minutes; the incubated mixture was removed and washed 5 times with diluted wash buffer.

The wells was struck sharply into absorbent material (paper towel) to remove residual wash buffer.

Substrate (A&B) was pipetted into all the wells , gently mixed for 5 seconds and incubated at room temperature for 20 minutes; the incubated mixture was removed and washed 5 times with diluted wash buffer.

Stop solution was added into the same coated wells, gently mixed for 30 seconds and the absorbance was read at 450nm using the microplate reader within 30 minutes.

Statistical Analysis

The outcomes of this study were statistically evaluated using SPSS software version 23.0, which is a statistical tool for social sciences. The variables were given a mean and standard deviation. Student's t-test statistical method was employed for comparisons.

Correlation coefficient was used to assess the association between two variables. Differences between three (3) age groups were assessed using Analysis of Variance (ANOVA) with Post Hoc (Non-parametric) comparison was used for intergroup variability.

The comparison was done at 95% confidence level, a p-value equal to or less than 0.05 ($p \leq 0.05$) were considered statistically significant.

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RESULT

Table 4.1: Comparison of Serum C-peptide levels among Breast Cancer Patients and the Control Group.

Table 4.1 shows that there was no significant difference in the serum levels of C-peptide and Alpha fetoprotein in women that had breast cancer compared to that of the women in the control group (p>0.05).

Table 4.1: Comparison of Serum C-peptide levels among Breast Cancer Patients and the Control Group

Parameters	**Control (n=45)**	**Test (n=45)**	**t-value**	***p*-value**
C-peptide (ng/mL)	1.54±1.36	1.40±1.67	0.444	0.658 (NS)

Keys: NS: Not significant (p>0.05) and n: Number of samples

Table 4.2: Comparison of Serum C-peptide levels across Different Age groups among Breast Cancer Patients.

Table 4.2 shows that there was no significant difference in the serum levels of C-peptide and alpha-fetoprotein levels across the age groups in breast cancer patients in NAUTH (p>0.05).

Table 4.2: Comparison of Serum C-peptide levels across Different Age groups among Breast Cancer Patients.

Age groups (years)	**C-peptide (ng/mL)**
26-34 (A)	2.60±3.45
35-43 (B)	0.63±0.31
44-52 (C)	1.45±1.63
≥53 (D)	1.47±1.10
F-value	2.015
p-value	0.127 (NS)

Table 4.3: Correlation of Serum C-peptide with the Anthropometric Characteristics in Breast Cancer Patients.

Table 4.3 shows that there was no significant correlation between age, body mass index, systolic and diastolic blood pressure with c-peptide and alpha fetoprotein (p>0.05).

Table 4.3: Correlation of Serum C-peptide with Anthropometric characteristics in Breast Cancer Patients.

Correlation	**C-peptide (ng/mL)**	
(n=45)	**r-value**	***p*-value**
Age	-0.084	0.584 (NS)
BMI	-0.103	0.499 (NS)

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SBP	0.051	0.738 (NS)
DBP	0.008	0.958 (NS)

Table 4.4: Correlation of Serum C-peptide with the Anthropometric Characteristics in Apparently Healthy Control Group.

Table 4.4 shows that there was no significant correlation between age, body mass index, systolic and diastolic blood pressure with c-peptide and alpha fetoprotein (p>0.05).

Table 4.4: Correlation of Serum C-peptide with the Anthropometric characteristics in Apparently Healthy Control Group.

Correlation	**C-peptide (ng/mL)**	
(n=45)	**r – value**	***p*-value**
Age	0.046	0.762 (NS)
BMI	0.000	1.000 (NS)
SBP	0.009	0.952 (NS)
DBP	-0.002	0.987 (NS)

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

Breast cancer remains one of the most prevalent malignancies affecting women worldwide, contributing significantly to morbidity and mortality. Despite advances in screening, diagnosis, and treatment, variability in disease outcomes persists, necessitating the search for reliable biomarkers to aid in prognosis and personalized therapy. This study evaluated the serum levels of C-peptide in breast cancer patients at Anambra State, Nigeria, with the aim of access the serum levels of the biomarkers and determining their potential role in breast cancer.

Findings from this study revealed that there was no significant difference in serum level of C-peptide between women diagnosed with breast cancer and their apparently healthy counterparts aged 18 to 60 years. This finding is consistent with a study by Jernström and Barrett-Connor (2009), which also reported no significant variations in C-peptide levels between breast cancer patients and healthy women. The discrepancy between these studies may be attributed to several factors, including differences in population characteristics, disease stage, metabolic influences, and lifestyle factors such as diet and physical activity (Fung *et al.*, 2012).

Findings from this study showed that within the breast cancer cohort there was no significant difference in serum C-peptide levels across different age groups. This finding contradicts the study by Li *et al.* (2020), which reported significantly higher C-peptide levels in women aged 50 to 60 years with breast cancer compared to younger patients.

Similarly, Shen *et al.* (2019) found an increased risk of breast cancer with higher C-peptide levels in Mexican women. The lack of an age-related trend in our study suggests that age alone may not be a primary determinant of C-peptide levels. Instead, other endocrine and metabolic factors, such as insulin resistance, hormonal fluctuations, and adiposity-related changes, could play a more substantial role (Vejrazkova *et al.*, 2020).

This study found no significant correlation between serum C-peptide levels with body mass index (BMI). These results contrast with the findings of Irwin (2005), who observed a positive correlation between C-peptide and BMI, suggesting a potential link between obesity, insulin resistance, and breast cancer risk.

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The absence of this association in our study may be attributed to physiological regulation mechanisms that maintain C-peptide homeostasis, even in individuals with varying BMI or blood pressure. Additionally, differences in insulin sensitivity, genetic predisposition, and dietary habits within our study population might have influenced C-peptide fluctuations, making it less directly correlated with anthropometric indices (Kurpiewska *et al.,* 2023).

Conclusion

This research started with the aim of assessing the role and serum levels of c-peptide in patients with breast cancer in Anambra state and from the findings of this systematic enquiry, there was no significant difference in the serum levels of c-peptide in patients with breast cancer compared to apparently healthy individuals that made up the control group also, there was no significant difference in the serum levels of c-peptide in patients with breast cancer across different age groups. Finally, there was no significant correlation between these biomarkers with age, body mass index and blood pressure.

Recommendations

I recommend that further research is required and should be carried out to determine whether C-peptide have subtype-specific prognostic value in breast cancer and to explore other metabolic pathways that may influence disease progression. Research should be carried out using a larger and more diverse sample. Longitudinal studies should be carried out tracking the levels of the biomarkers with the progression of breast cancer.

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