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*Research Article*

# Immunohistochemical Correlation Between Vitamin D Receptor and Human Epidermal Growth Factor Receptor (HER-2) in Breast Cancer

***Fasogbon S.A[1], Okoye J.O[2] and Okorie N[3]**

[1]*Public Health In-vitro Diagnostic Control Laboratory, Medical Laboratory Science Council of Nigeria. 8, Harvey Road, Yaba, Lagos, Nigeria*

[2]*Medical Laboratory Science Department, School of Public and Allied Health, Babcock University, Ilishan-Remo, Ogun State, Nigeria*

[3]*N.K.S.T. Len Gebrielse' School of Medical Laboratory Science Mkar, Benue State, Nigeria*

**ABSTRACT**

The vitamin D receptor (VDR) gene is expressed in breast tissue and known to modulate the rate of cell proliferation; HER-2 proteins are receptors on breast cells which normally help control how a healthy breast cell grows. This study was carried out to determine the Immunohistochemical Correlation between the Expression of Vitamin D Receptor (VDR) and Human Epidermal Growth Factor Receptor (HER-2) in Invasive Ductal Carcinoma (IDC) tissues. A total number of fifty-six (56) archived female breast Invasive Ductal Carcinoma tissue blocks were used. The tissue blocks were sectioned at not more than 2µm each. Haematoxylin and Eosin staining method and immunohistochemical staining technique using VDR and HER-2 antibodies were done and the results were correlated. The results show that there is a significant difference ($P<0.05$) found comparing the immunohistochemical expression of VDR with HER-2 in IDC tissues. VDR antibody cannot be used in substitute to HER-2 antibody in the immunohistochemical diagnosis of Invasive ductal carcinoma (IDC) of the breast. However, VDR's strong positive expression in IDC tissues may indicate its links with breast cancer. Therefore, VDR may be recommended as an additional antibody in the diagnosis and breast cancer therapeutics

**Keywords:** Vitamin D receptor, Human Epidermal Growth factor receptor (HER-2), Invasive ductal carcinoma, Breast Cancer.

*Author for correspondence: *E-mail*: *samfash4best@gmail.com ;* +2348032229904

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## INTRODUCTION

Invasive ductal carcinoma (IDC), also known as infiltrating ductal carcinoma, is cancer that began growing in the duct and has invaded the fatty tissue of the breast outside of the duct. IDC is the most common form of breast cancer, representing 80 percent of all breast cancer diagnoses (Johns Hopkins, 2016). Breast cancer is the most frequent cancer among women, being a heterogeneous disease, with distinct morphologies, metastatic behaviour and therapeutic response (Ricardo et al., 2011). Approximately, 90% of breast cancer deaths are caused by local invasion and distant metastasis of tumor cells (Yifau and Binhua, 2011). According to (Viale, 2012), different types of this neoplasm exhibit variable histopathological and biological features, different clinical outcome and different response to systemic interventions. In fact, global gene-expression analyses have provided an appealing molecular classification for breast carcinomas, which is highly associated with patients' prognosis (Sotiriou et al., 2003). In the last decade; a major effort has been made to better inform the choice of the systemic treatment for breast cancer patients.

The calcitriol receptor, also known as the vitamin D receptor (VDR) and also known as NR1I1 (nuclear receptor subfamily 1, group I, member 1), is a member of the nuclear receptor family of transcription factors (Hosoi, 2002). Upon activation by vitamin D, the VDR forms a heterodimer with the retinoid-X receptor and binds to hormone response

elements on DNA resulting in expression or transrepression of specific gene products. The VDR not only regulates transcriptional responses but also involved in microRNA-directed post transcriptional mechanisms (Uitterlinden et al., 2004). In humans, the vitamin D receptor is encoded by the VDR gene (Norman, 2007). Glucocorticoids are known to decrease expression of VDR, which is expressed in most tissues of the body and regulate intestinal transport of calcium, Iron and other minerals (Bollag, 2007). Also, it has recently been identified that VDR as an additional bile acid receptor alongside FXR and may function to protect gut against the toxic and carcinogenic effects some endobiotics (Salashor and Woodgett, 2002). Many studies have shown that there is a link between vitamin D and breast cancer. Women who have breast cancer tend to have low levels of vitamin D in their body. Researchers have found how vitamin D might have a role in breast cancer. Vitamin D receptors are found on the surface of a cell where they receive chemical signals. By attaching themselves to a receptor, these chemical signals direct a cell to do something, for example to act in a certain way, or to divide or die. There are vitamin D receptors in breast tissue, and vitamin D can bind to these receptors. These can oncogenes to die or stop growing, and can stop the cancer cells from spreading to other parts of the body. Therefore, it is thought that vitamin D may help in protecting against breast cancer, by making cells in the breast smarter. However, the relationship between breast cancer and vitamin D is complex, not fully understood, and is still being studied (Rose et al., 2013; Wang et al., 2013; Welsh, 2012).

Breast cancer is the predominant malignancy where oncologists use predictive markers clinically to select treatment options, with steroid receptors having been used for many years. Immunohistochemistry has taken over as the major assay method used for assessing markers (Walker, 2007). The advent of molecular technology has incorporated new biomarkers along with immunohistochemical and serum biomarkers. Immunohistochemical markers [Estrogen receptor (ER), Progesterone receptor (PR), and Human epidermal growth factor receptor 2 (HER-2)] are often used to guide treatment decisions, to classify breast cancer into subtypes that are biologically distinct and behave differently, and both as prognostic and predictive factors (Walker, 2007).

The HER2 gene makes HER-2 proteins. HER2 proteins are receptors on breast cells. Normally, HER2 receptors help control how a healthy breast cell grows, divides, and repairs itself. But in about 25% of breast cancers, the HER2 gene doesn't work correctly and makes too many copies of itself (known as HER2 gene amplification) (Breastcancer.org, 2017). HER2-positive breast cancer is a breast cancer that tests positive for a protein called human epidermal growth factor receptor 2 (HER2), which promotes the growth of cancer cells (Mayoclinic, 2017). VDR polymorphisms are associated with breast cancer risk and may be associated with disease progression (Guy et al., 2004). The vitamin D receptor (VDR) gene is expressed in breast tissue and known to modulate the rate of cell proliferation (Buyru et al., 2003). However, the correlation between VDR and her2 has not been confirmed by any study. This research therefore correlates the immunohistochemical expression of VDR with HER-2 in IDC tissues.

## MATERIALS AND METHODS

### Area of Study

This study was carried out at Department of Histopathology, National Hospital Abuja, FCT, Nigeria. The Hospital serves most of the states of Nigeria and therefore serving a significant population of the region.

### Ethical Standards

The appropriate ethics committee approved all studies and carried out in accordance with 1964 Declaration of Helsinki ethical standards**.** All persons gave their informed consent prior to their inclusion in the study.

### Sample Size

A total of fifty-six (56) samples were used. Sample size was determined using a formula by (Naing *et al.,* 2006).

### Sample Collection/Histopathological Procedures

Paraffin tissue blocks diagnosed of invasive ductal carcinoma of the female breast were used. The tissue blocks were sectioned at not more than 2µm each. From each block were obtained five sections in which one (1) section was used for Haematoxylin and Eosin staining technique while two (2) sections were treated each with VDR and HER-2 antibodies, while the other two (2) sections were used as negative and positive control.

### Haematoxylin and Eosin Staining Technique

The sections were taken to water, stained using Harris Haematoxylin for 5minutes, washed in tap water then differentiated in 1% acid alcohol for few seconds. They were washed in tap water then blued in tap water for 10minutes. The sections were then counterstained in 1% Eosin for 1minutes. They were then washed in tap water, dehydrated, cleared and mounted using DPX (Avwioro, 2014).

### Immunohistochemical Technique

The method used is the Avidin Biotin Complex (ABC) method and the antibodies used are manufactured by Novocastra. The antibody dilution factor used was 1:100 dilutions for all the antibody markers.

The processed tissues were sectioned at 2µm on the rotary microtome and placed on the hot plate at $70^0$C for at least 1hour. Sections were brought down to water by passing them in 2 changes of Xylene, then 3 changes of descending grades of alcohol and finally to water. Antigen retrieval was performed on the sections by heating them on a Citric Acid solution of pH 6.0 using the Microwave at $100^0$C for 15minutes. The sections were equilibrated gradually with cool water to displace the hot Citric Acid for at least 5min. Peroxidase blocking was done on the sections by covering them with 3% hydrogen peroxide ($H_2O_2$) for 15min. Sections were washed with PBS and protein blocking was performed using avidin for 15min. Sections were washed with PBS and endogenous biotin in tissue was blocked using biotin for 15min. After washing with PBS sections were incubated with the respective diluted primary antibody antibody diluted 1:100 for 60 min. Excess antibodies were washed off with PBS and a secondary antibody (link) was applied on section for 15min.

Sections were washed and the (label, in this case which is the Horseradish Peroxidase HRP) was applied on the sections for 15min. A working DAB solution is made up by mixing 1 drop (20µl) of the DAB chromogen to 1ml of the DAB substrate. This working solution was applied on sections after washing off the HRP with PBS for at least 5min. The brown reaction began to appear at this moment especially for a positive target. Excess DAB solution and precipitate were washed with water. Sections were counterstained with Haematoxylin solution for at least 2min and blued briefly. Sections were dehydrated in alcohol, cleared in Xylene and mounted in DPX (Marc, 2009).

**Immunohistochemical Analysis**

Cells with specific brown colours in the cytoplasm, cell membrane or nuclei depending on the antigenic sites were considered to be positive. The Haematoxylin stained cells without any form of brown colours were scored negative. Nonspecific binding/brown artifacts on cells and connective tissue were disregarded (Marc, 2009).

**Statistical Analysis**

Photomicrograph was basically used for correlating the expression and where necessary, Paired T-test statistics method was used to analyse the data generated.

**RESULTS**

A total of fifty-six (56) tissue blocks already diagnosed as invasive ductal carcinoma of the female breast (Age mean=46.4) were used for the study. The results ae presented in Tables 1 & 2, and in Fig. 1-7.

**Table 1:**
**Expression of VDR and HER-2 in invasive Ductal Carcinoma (IDC)**

| Parameter | VDR | HER-2 |
|---|---|---|
| Postive | 37 | 15 |
| Negative | 19 | 41 |
| **Total** | 56 | 56 |

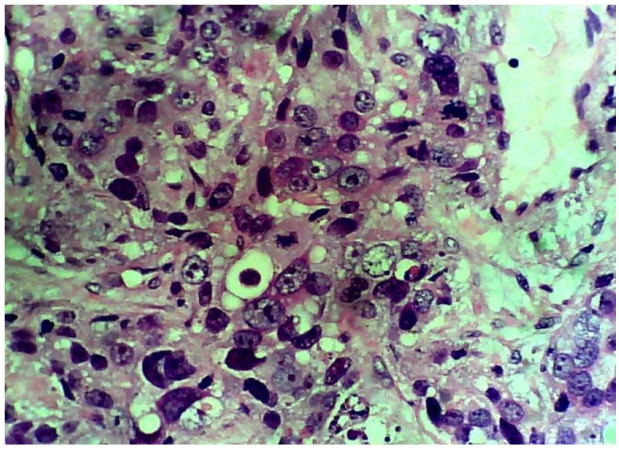

**Fig. 1**:
Invasive Ductal Carcinoma (IDC) of the breast showing proliferation of epithelial cells appearing as atypical cells with marked nuclear enlargement and hypercromasia (H and E; x400)

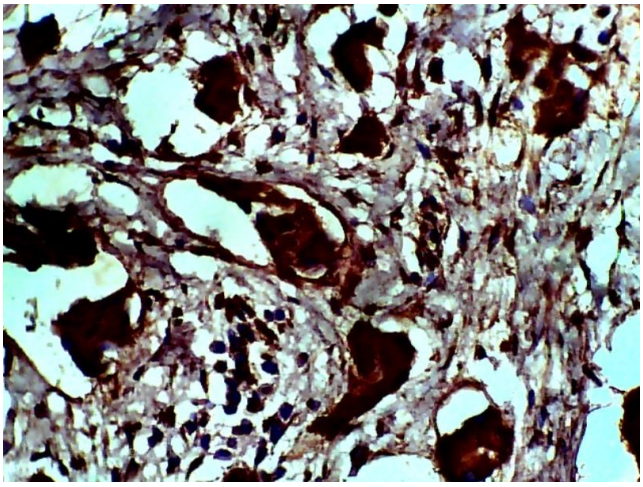

**Fig. 2**:
Invasive Ductal Carcinoma (IDC) tissue showing Positive expression of Vitamin D Receptor (VDR) (x400)

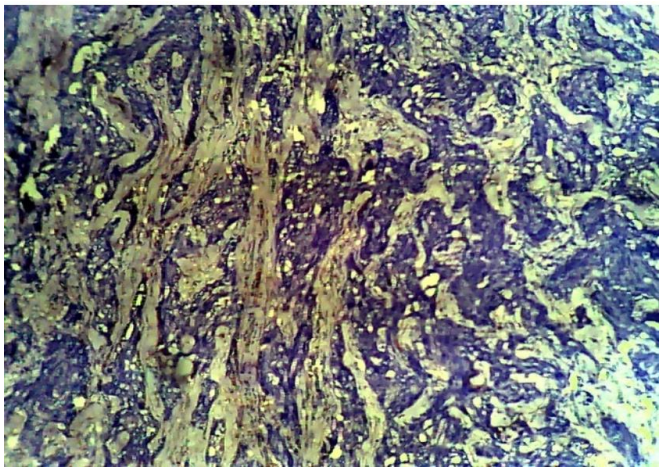

**Plate 3**:
Invasive Ductal Carcinoma (IDC) tissue showing Negative expression of Vitamin D Receptor (VDR) (x100)

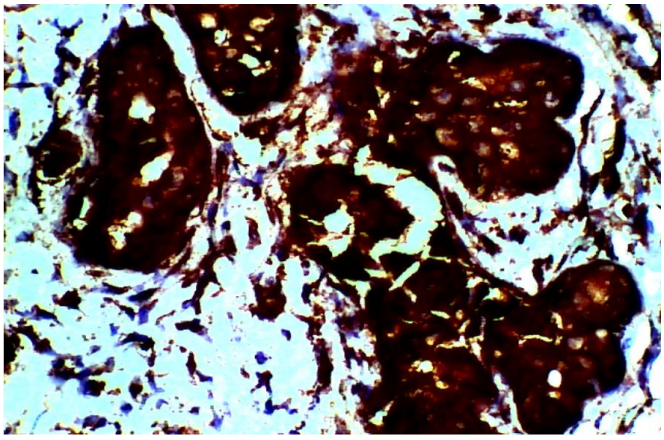

**Plate 4**:
Invasive Ductal Carcinoma (IDC) tissue showing Positive expression of HER-2 x400

**Table 2:**
Correlation of Immunohistochemical Expression between VDR and HER-2 In IDC tissues (Paired t-Test)

| | **Paired Samples Statistics** | Mean | N | SD | SEM |
|---|---|---|---|---|---|
| Pair 1 | HER-2 | 1.55 | 56 | 0.502 | 0.067 |
| | VDR | 1.34 | 56 | 0.478 | 0.064 |
| **Paired samples Correlation** | | **N** | **Correlation** | **sig** | |
| | HER-2 VDR | 56 | .008 | .956 | |

*The mean ± SEM are 0.393 ± 0.087, therefore there is a significant difference/relationship between HER2 and VDR at a significant level (P)=0.001<0.05, t55= 4.511, Pearson(r)= 0.956*

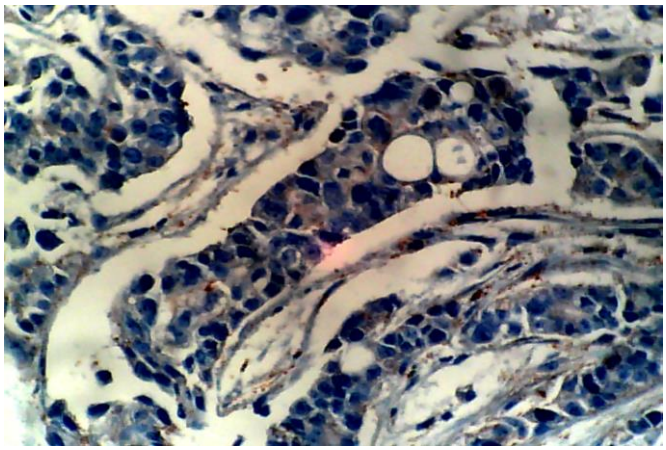

**Plate 5**
Invasive Ductal Carcinoma (IDC) tissue showing Negative expression of HER2 (x400)

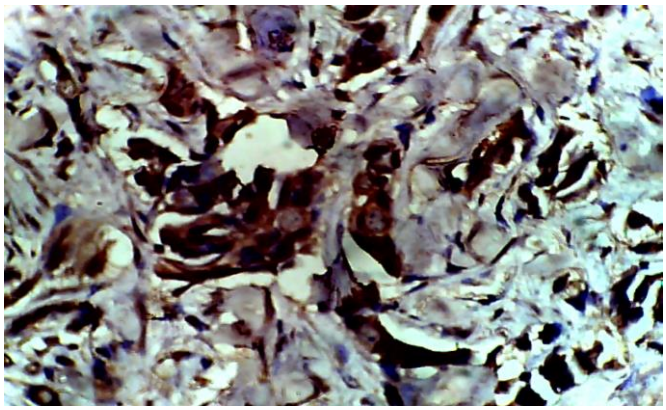

**Plate 6:**
Positive Control x400

## DISCUSSION

There were significant differences between VDR with HER-2 expressions in IDC tissues which indicate that VDR cannot be used over HER-2 in the immunohistochemical diagnosis of IDC. This result is supported by earlier related study done by (Friedrich et al., 2002) on VDR expression analyzed immunohistochemically in breast cancer patients who reported that no statistically significant correlations were found comparing VDR expression with expression of estrogen receptors (ER) or progesterone receptors (PR), even with the proliferation marker Ki-67, with the tumor suppressor gene p53 or with the S-phase index. The findings indicate that VDR protein expression is not a prognostic factor in breast cancer (Friedrich et al., 2002).

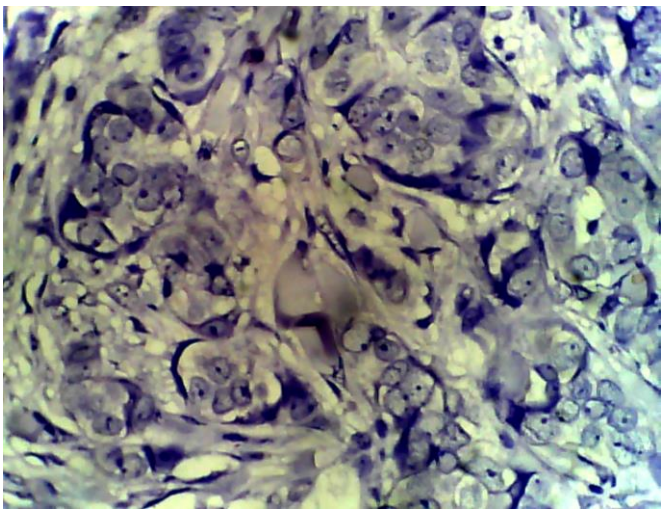

**Plate 7:**
Negative Control x400

VDR shows the highest and strong positive expression on IDC tissues in this research which could indicate a link between Vitamin D receptor and breast cancer. This support a study carried out in which a strong VDR immunoreactivity was observed in breast cancer specimens, supporting the body of evidence that breast cancer may be a target for therapeutically applied vitamin D analogues (Friedrich et al., 2002; Fasogbon et al., 2017).

This also support a study carried out that said; there are vitamin D receptors in breast tissue, and vitamin D can bind to these receptors. This can cause oncogenes to die or stop growing, and can stop the cancer cells from spreading to other parts of the body. Therefore, it is thought that vitamin D may help in protecting against breast cancer (Rose et al., 2013).

On the basis of this study and review of relevant literature it is concluded that VDR has no statistically significant correlations when compared with HER-2 antibodies; But VDR the highest rate positivism in IDC tissues can indicate it links with breast cancer. Therefore, VDR can be recommended as an additional antibody in the diagnosis and breast cancer therapeutics.

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