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Immunohistochemical Correlation between the Expression of Vitamin D Receptor (VDR) and Triple Negative Invasive Ductal Carcinoma Tissues

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Abstract

Aim: The study was carried out to determine the Immunohistochemical Correlation between the Expression of Vitamin D Receptor (VDR) and Triple Negative Invasive Ductal Carcinoma (IDC) tissues Materials and Method: Twenty two (22) cases of archived female breast Invasive Ductal Carcinoma tissue blocks that are Negative to ER, PR, and HER-2 (Triple Negative) were used. The tissue blocks were sectioned at not more than 2µm each. Haematoxylin and Eosin staining method and immunohistochemical staining technique using VDR antibodies were done and the results were correlated. Result and Discussion: The results show that there is a significant difference ($P<0.05$) found comparing the immunohistochemical expression of VDR with Triple Negative IDC tissues but strong positivity is shown in VDR positive IDC tissues. Conclusion: There is a statistically significant difference found in the expression of VDR with Triple negative IDC tissues; therefore VDR cannot be use as substitute in cases of triple negative IDC tissues but can be an additional antibody and of therapeutic target in breast cancer.

Keywords: Vitamin D receptor (VDR), Triple Negative, Invasive ductal carcinoma (IDC), Breast Cancer

1. Introduction

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 [1]. 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 [1]. A diagnosis of triple negative breast cancer means that the three most common types of receptors known to fuel most breast cancer growth-estrogen, progesterone, and the HER-2/neu gene-are not present in the cancer tumor. This means that the breast cancer cells have tested negative for hormone epidermal growth factor receptor 2(HER-2), estrogen receptors (ER), and progesterone receptors (PR). Since the tumor cells lack the necessary receptors, common treatments like hormone therapy and drugs that target estrogen, progesterone, and HER-2 are ineffective [2]. Triple negative breast cancer occurs in about 10-20% of diagnosed breast cancers and is more likely to affect younger people, African Americans, Hispanics, and/or those with a BRCA1 gene mutation. Triple negative breast cancer can be more aggressive and difficult to treat. Also, the cancer is more likely to spread and recur [2]. 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 [3]. Breast cancer is the most frequent cancer among women, being a heterogeneous disease, with distinct morphologies, metastatic behaviour and therapeutic response [4]. Approximately, 90% of breast cancer deaths are caused by local invasion and distant metastasis of tumor cells [5]. According to [6], 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 [7].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 [8]. 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 [9]. In humans, the vitamin D receptor is encoded by the VDR gene [10]. 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 [11]. 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 [12]. 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 [13,14,15]. The researcher therefore, has set out to correlate the immunohistochemical expression of VDR with Triple negative IDC tissues.

2. Research Methodology

2.1 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.

2.2 Ethical Consideration

Approval for this research work was obtained from the Research Ethics Committee of National Hospital Abuja, FCT, Nigeria; in line with that set by World Health Organization.

2.3 Sample Size

Twenty two (22) cases of Triple negative Invasive ductal carcinoma tissue samples were obtained.

2.4 Sample Collection/Histopathological Procedures

Paraffin tissue blocks diagnosed of invasive ductal carcinoma (IDC) of the female breast and are negative to ER, PR, and HER-2 (Triple negative) of the female breast were used.

The tissue blocks were sectioned at not more than 2µm each. Four (4) sections were obtained from each block from which one (1) section was used for Haematoxylin and Eosin staining technique while one (1) section was treated with VDR antibodies, while the other two (2) sections were used as negative and positive control.

2.5 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 [16].

2.6 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^{o}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 (H2O2) 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 [17].

2.7 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. Non specific binding/brown artifacts on cells and connective tissue were disregarded [17].

2.8 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.

3. Results

Twenty two (22) cases of triple negative tissue blocks already diagnosed as invasive ductal carcinoma of the female breast (Age mean=46.4) were used for the study. The results were presented in table and figures below:

Table 1: Correlation of Immunohistochemical Expression of VDR with triple negative (ER, PR and HER2) IDC tissues (paired t-test)

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	VDR	2.00	22	< .001	.000
	ER	1.27	22	.456	.097
Pair 2	VDR	2.00	22	< .001	.000
	PR	1.27	22	.456	.097
Pair3	VDR	2.00	22	< .001	.000
	HER2	1.27	22	.456	.097

The Mean ± SEM are 0.727 ± 0.097 respectively, therefore there is a significant difference (No significant correlation) between NEGATIVE RESULTS OF ER, PR and HER2 over VDR at a significant level (P) = 0.001<0.05, t_{21}= 7.483, RESPECTIVELY.

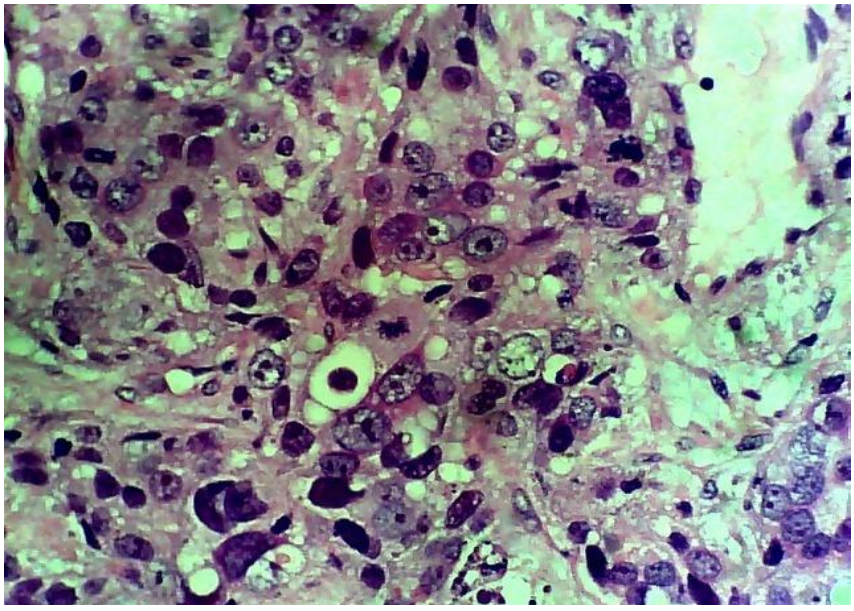

Figure 1: Invasive Ductal Carcinoma (IDC) of the breast showing proliferation of epithelial cells appearing as atypical cells with marked nuclear enlargement and hypercromasia (H and E; x400)

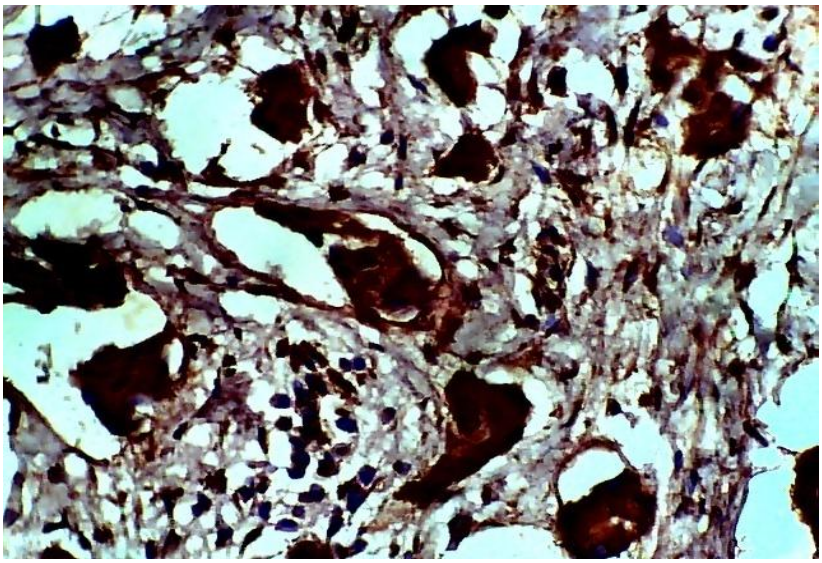

Figure 2: Invasive Ductal Carcinoma (IDC) tissue showing Positive expression of Vitamin D Receptor (VDR) (x400)

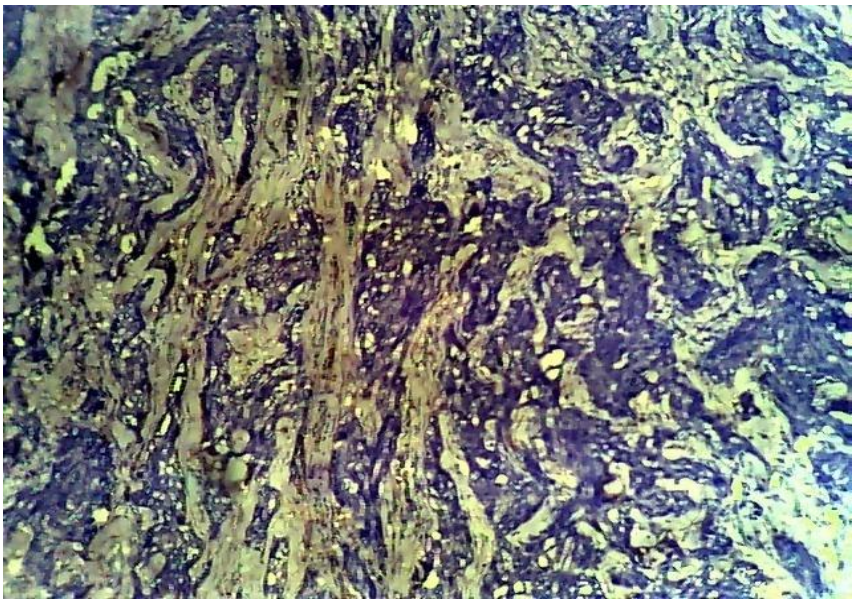

Figure 3: Invasive Ductal Carcinoma (IDC) tissue showing Negative expression of Vitamin D Receptor (VDR) (x100)

4. Discussion

There is a significant difference found; comparing VDR expression with Triple negative IDC tissues which indicate that VDR cannot be used as a replacement in triple negative IDC cases; this result is supported by earlier work done by [18] 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 [18]. VDR shows 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 [19]. 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 [13].

As at the period of this research, many studies has reveals the relationship of VDR with breast cancer which gives strength to this study; but the limitation encountered was that, no literature was found correlating immunohistochemical expression of VDR with ER in Breast cancer.

5. Conclusion

On the basis of this study and review of relevant literature, VDR has statistically significant difference when compared with triple negative IDC tissues, therefore VDR cannot be use as a substitute in cases of triple negative IDC tissues; but VDR can be use as an additional antibody in immunohistochemical diagnosis and be of therapeutic target in breast cancer.

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