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MIEN OF RETINOBLASTOMA PROTEIN (pRb) IN GRADES OF CERVICAL LESION

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ABSTRACT

The retinoblastoma protein (pRb); gene appellation (shortened *RB* or *RB1*) is a tumor suppressor protein that stands dysfunctional in numerous major malignancies. Cervical cancer is another utmost common cause of cancer-related death globally between women folk. This study was aimed at investigating the expression of retinoblastoma protein (pRb) in grades of cervical lesion. Two hundred (200) cases of archived formalin fixed paraffin embedded (FFPE) tissues of already diagnosed cervical lesions and cervical carcinoma tissue blocks were selected for this study. Sections of 3µ thickness were cut for immunohistochemical staining technique. The immunohistochemical staining was semi-quantitatively scored based on percentage of cells that stained positive and the intensity of the staining. Photomicrograph was basically used for analyzing the expression, and comparative analysis of the data generated from the expression was statistically done. Frequency of data was calculated using Chi-square (x^2). The expression of retinoblastoma protein (pRb) was found to be 95.3% in cervical intraepithelial neoplasia grade I (CIN I) cases, 100% in CIN II cases, 85.4% in CIN III cases, and 60.8% in squamous cell carcinoma (SCC) cases. The expression of pRb in CIN I, II, and III as well as SCC significantly reduced as the CIN cases matured to malignancy ($p<0.05$) with CIN III been observed with the least expression of pRb among CIN cases. The down-regulation of pRb observed in this study may indicate mutation of RB gene in cervical lesions and could be involved in cervical carcinogenesis.

Keywords: Cervical, dysplasia, immunoreactivity, retinoblastoma, protein.

INTRODUCTION

The retinoblastoma (RB) tumor suppressor protein plays a pivotal role in the control of cell cycle, terminal differentiation, and various other biological events (Dyson, 2016). RB is genetically or functionally inactivated in numerous human cancers, including retinoblastoma, small cell lung cancer (SCLC), prostate cancer, and breast cancer (Shunsuke et al. 2020). The retinoblastoma susceptibility gene (*RB1*) was the first tumor suppressor gene to be molecularly defined and the retinoblastoma protein (pRb) which is its gene product regulates transcription and is a negative regulator of cell proliferation (Dyson, 2016). The canonical pathway whereby RB exerts its tumor suppressive activity entails the formation of a transcriptional repression complex with E2F transcription factors and various chromatin modifiers, such as histone deacetylases (HDACs). This complex orchestrates the G1/S transition during cell cycle progression primarily by controlling E2F target genes (Dyson, 2016; Kitajima and Takahashi, 2017).

The *Rb1*-encoded protein (pRb) is well known as a general cell cycle regulator, and this activity is critical for pRb-mediated tumor suppression (Goodrich, 2006). Better insight into pRb-mediated tumor suppression and clinical exploitation of pRb as a therapeutic target will require a global view of the complex interdependent network of pocket protein complexes that function simultaneously within given tissues (Goodrich, 2006). Most mitogenic signals commonly merge on the transcriptional up regulation of D-type cyclins and then stimulate cyclin-dependent kinases, including CDK4/6. D-type cyclin-CDK4/6 complexes

have been proposed to promote mono-phosphorylation on RB, which allow it to exert early G1 functions by starting the release of E2Fs. E2F target genes, including cyclin E and A, in cooperation with CDK2 or CDK1, are responsible for full phosphorylation of RB at 13 remaining sites. This allows cells to enter the S and M phases (Rubin, 2013; Dyson, 2016; Sanidas et al. 2019). The fact that genetic and/or epigenetic aberrations of the components in the RB pathway tend to be mutually exclusive in the patients might implicate a linearity of the RB pathway (Shunsuke et al. 2020).

Cervical cancer is a major gynaecological cancer which encompasses hysterical cell division and tissue invasiveness of the female uterine cervix (Dasari et al. 2015). Cervical cancer is the second most common cause of cancer-related death among women worldwide, with over 500,000 new cases diagnosed annually and 50% mortality rate in Asia (Daniyal et al. 2015). Cancer cells can disrupt the immune response through the over expression of inhibitory molecules like PD-L1 (Patel and Kurzrock, 2015) or the loss of expression of stimulatory molecules like CD40L. Tumors can evade immune surveillance even in the presence of tumor antigens because the immune cells may not receive adequate signals for activation and proliferation or are suppressed by inhibitory checkpoint proteins (Whiteside, 2006).The decision to commit to a new round of cell division occurs when the cell activates cyclin-CDK-dependent transcription which promotes entry into S phase (*Bertoli et al.2013*). During early G1, the transcriptional repressors Rb (retinoblastoma), p107 and p130, known as pocket proteins, bind to the E2F transcription factors to prevent G1-to-S transition. Rb binds and represses activator E2F transcription factors (E2F1-3), while p107 and p130 bind E2F4 and E2F5 respectively to form complexes which repress transcription of G1-to-S promoting factors (proteins) (*Bertoli et al. 2013*). This study therefore investigated the expression of retinoblastoma protein **(pRb**) in grades of cervical lesion.

MATERIALS AND METHODS

Study Population

This study was conducted on tissue blocks from women that had CIN I, II, III and cancer of the cervix in Lagos University Teaching Hospital, Lagos. The age of the patients ranged from 28 to 64years. The duration of the tissue blocks used was from 2002-2016.

Ethical Consideration

Ethical approval for this study was obtained from the Ethics and Research Committees of Lagos University Teaching Hospital, Lagos

Specimen collection

Two hundred (200) cases of archived formalin fixed paraffin embedded (FFPE) tissues of already diagnosed cervical lesions and cervical carcinoma tissue blocks were selected for this study.

Histopathological Procedures

Each formalin fixed paraffin wax embedded tissue was sectioned to harvest tissue areas with the highest lesion. Sections of 3µ thick were cut for immunohistochemical staining technique. Four sections were obtained from each block of cervical intraepithelial neoplasia grade I (CIN I), CIN II, CIN III and cervical carcinoma; from which one section was used for haematoxylin and eosin staining technique, one section was treated with retinoblastoma protein (pRb) while the other section served as negative and positive controls. Haematoxylin and eosin method was carried out to confirm earlier diagnosis before proceeding to the immunohistochemical analysis.

Haematoxylin and Eosin Staining Technique (Avwioro, 2014) was used to stain the sections and Immunohistochemical Technique was performed (Marc, 2009).

Control for Immunohistochemistry (IHC):

Positive control sections for the marker was obtained from tissues that are known to express the antigen.In negative control, tissues that are kown not to express the antibody marker was used, while in the reagent negative controls, pRb antibody that is being tested was omitted.

Reading of Immunohistochemistry results

Cells with specific brown colours in the cytoplasm, cell membrane or nuclei depending on the antigenic sites were observed. The haematoxylin stained cells without any form of

brown colours were scored negative. Non-specific binding/brown artifacts on cells and connective tissue was disregarded (Marc, 2009). Immunoreactive Scoring System by Klein et al. (1999) was used.

Statistical Analysis

Photomicrograph was basically used for analyzing the expression and comparative analysis of the data generated from the expression of the pRb protein were statistically analyzed by the statistical data software SPSS version 16 (SPSS Inc. Chicago, Illinois) for windows. Descriptive statistical analysis was also used to analyze some of the data.

RESULTS

The tables below summarize the expression of retinoblastoma protein (pRb) in cervical intraepithelial neoplasia grade I (CIN I), CIN II, CIN III, and Cervical squamous cell carcinoma (SCC).

Retinoblastoma protein (pRb) was highly expressed in 48.9% of the overall CIN diagnosis but in 10.1% of SCC cases. Low expression of pRb was observed in 46.5% and 50.7% of overall CIN and SCC cases respectively. pRb expression was significantly reduced as the dysplasia progresses ($p<0.05$) (Table 1).

Table 1: Expression of pRb in Overall CIN and SCC

Case	No. Tested	Positive Expression		Negative Expression
		High(%)	Low(%)	(No expression)(%)
CIN	**131**	64(48.9)	59(46.5)	8(4.6)
SCC	**69**	7(10.1)	35(50.7)	27(39.2)

*$p<0.05$

pRb was most expressed among participants diagnosed of CIN II (53.2%), followed by CIN I(48.8%), CIN III (43.9%) whereas those diagnosed of SCC had the least expression of pRb (10.1%). The expression of pRb in CIN I, II, and III as well as SCC significantly reduced as the CIN cases matured to malignancy ($p<0.05$) with CIN III been observed with the least expression of pRb among CIN cases. Generally, the SCC revealed the least expression of pRb (10.1%) in all dysplasia cases (Table 2).

Table 2: Expression of pRb in CIN I, CIN II, CIN III, and Squamous cell carcinoma of the Cervix

Case	No. Tested	Positive Expression		Negative Expression
		High (%)	Low (%)	(No expression)(%)
CIN I	**43**	20(46.5)	21(48.8)	2(4.7)
CIN II	**47**	22(46.8)	25(53.2)	0
CIN III	**41**	17(41.5)	18(43.9)	6(14.6)
SCC	**69**	35(50.7)	7(10.1)	27(39.2)

CIN - Cervical Intraepithelial Neoplasia;
SCC - Squamous cell carcinoma of the Cervix.

*$p<0.05$

In this study, strong expression of pRb was observed in CIN I which was demonstrated endogenously with the brownish pigmentation on the section (Fig. 1).

Moderate expression of pRb was observed in CIN II that was demonstrated endogenously with the brownish pigmentation on the section (Fig. 2).

Mild expression of pRb was observed in CIN III which was demonstrated endogenously with the brownish pigmentation on the section (Fig. 3).

Low expression of pRb was indicated in SCC which was demonstrated endogenously with the brownish pigmentation on the section (Fig. 4).

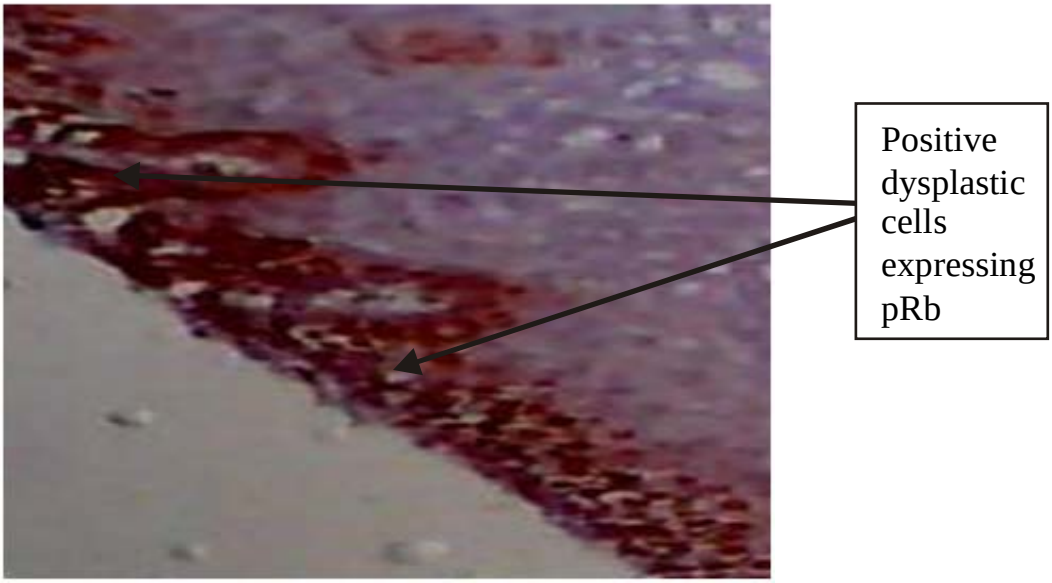

Fig. 1: Immunohistochemistry of Strong Expression of pRb in CIN I (mag x400)

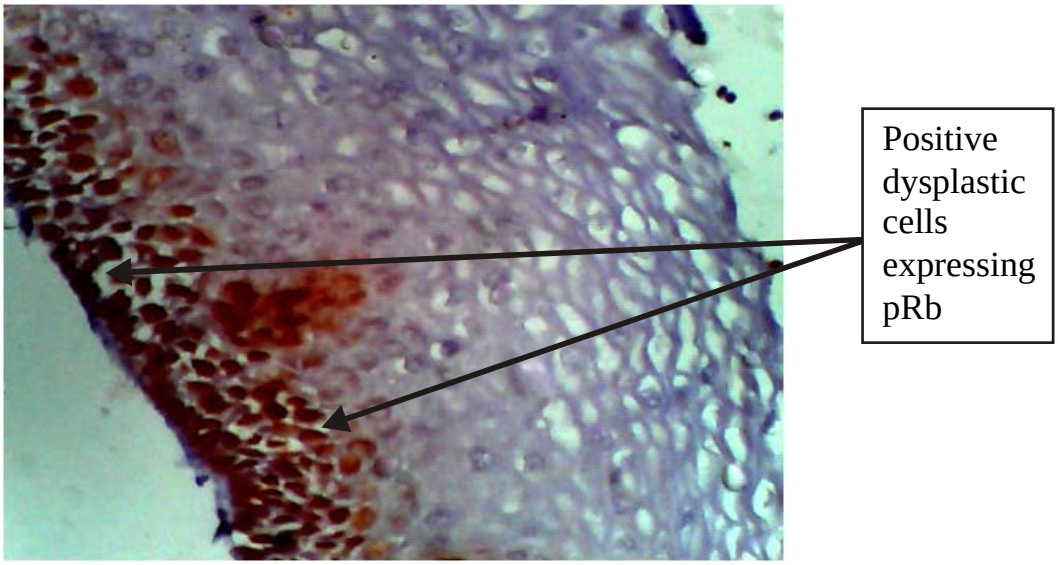

Fig. 2: Immunohistochemistry of Moderate Expression of pRb antibody in CIN II (mag x400)

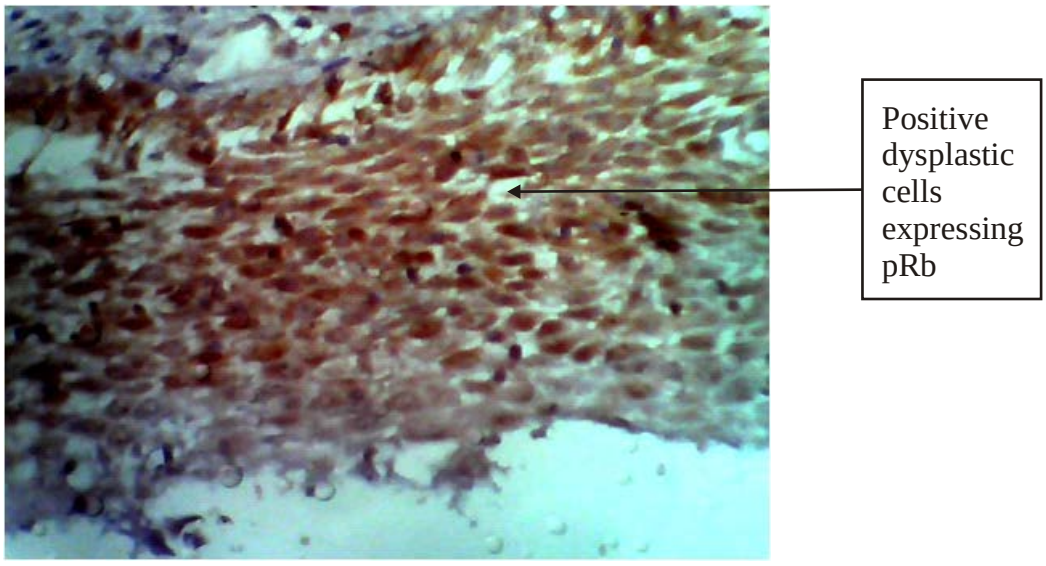

Fig. 3: Immunohistochemistry of Mild Expression of pRb antibody in CIN III (mag x400)

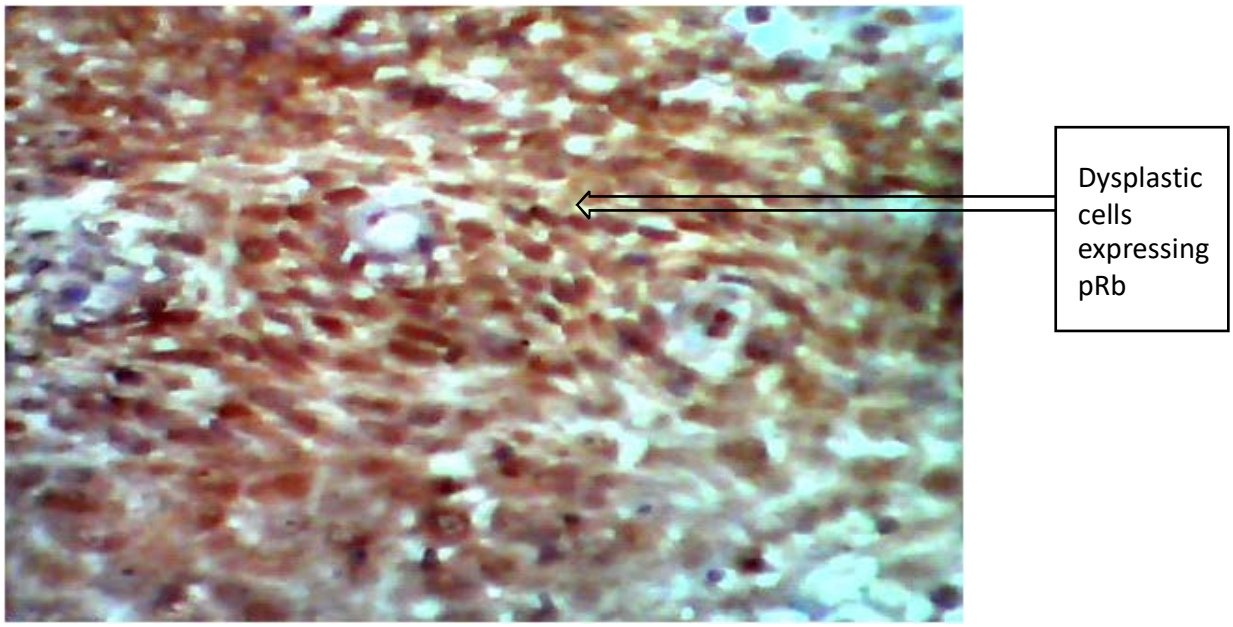

Fig. 4: Immunohistochemistry of Low Expression of pRb antibody in SCC (mag x400)

DISCUSSION

The RB tumor suppressor pathway has been extensively studied (Dick et al. 2018). The retinoblastoma protein (pRb) is a cell cycle regulator predominantly known as cell cycle repressor deactivated in most human cancers. Loss of pRb function results from mutations in the gene coding for pRb or for any of its upstream regulators (Sosa-García et al. 2010). The inactivation of RB1 through somatic mutation, deletion, or epigenetic silencing in various cancers has been reported. Hypophosphorylated pRB binds to and represses the transcriptional activity of E2F family members, which control the expression of genes necessary for cell cycle progression (Wang et al. 2018).

In this study, the expression of retinoblastoma Protein (pRb) was observed to be 95.3% in CIN I cases, 100% in CIN II cases, 85.4% in CIN III cases, and 60.8% in SCC cases. Low level expression of pRb was observed to be more frequent in SCC cases (50.7%) followed by CIN II (46.8%), CIN I (46.5%), and CIN III (41.5%). This study showed pRb immunoreactivity to be frequent in majority of the CIN and SCC cases which is comparable to the findings by several authors that reported ubiquitous expression of pRb in normal or cancerous tissues (Horowitz et al. 1990; Furukawa et al. 1991; Cordon-Cardo and Richon, 1994; Salcedo et al. 2002); Some studies that were also done on normal uterine-cervix tissue shows that pRb is expressed in mature and differentiated cells, in the basal third epithelium in 90% of normal/reactive atypia or in the scattered nuclei of normal cells in all cases tested (Benedict et al. 1990; Cance et al. 1990; Cordon-Cardo et al. 1992).

This study observed that pRb immunoreactivity in SCC was frequently lower than in CIN which is in agreement with the report of some workers (Ludlow et al. 1993; Boyer et al. 1996) suggesting that the down regulation of retinoblastoma (RB) gene could be involved in cervical carcinogenesis. The low expression of pRb may also be linked to inactivation of pRb which is as a result of complex formation with high risk HPV E7 oncoprotein and its degradation, down regulation mechanisms (Boyer et al. 1996; Whyte et al. 1988; Dyson et al. 1989). The heterogeneous pRb immunostaining observed in CIN stages as compared to SCC in this study is similar to that reported by Salcedo et al. (2002) which also observed heterogeneous pRb immunostaining during the different stages of cervical carcinogenesis and suggest that this staining pattern could be a common feature implicated in pathological process of uterine-cervix carcinoma. This study also showed a positive statistical correlation between the degree of dysplasia and the degree of pRb

expression in all the cases which indicates that as degree of cervical dysplasia increases (from premalignant to malignant lesion), there will be gradual decrease in pRb expression; this findings on CIN and SCC can be related to the suggestion of some authors that pRb negative tumours might be clinically more aggressive and with a poorer prognosis than those tumours containing a variable pRb expression (Benedict et al. 1990; Hanson et al. 1994; Harbour et al. 1988). In support of this possibility, some studies also reported that high RB gene expression inhibits tumour cell invasion *in vitro* (Li et al. 1996).

CONCLUSION

Retinoblastoma protein (pRb) expression revealed a progress in the degree of cervical dysplasia (premalignant to malignant lesion), with a corresponding gradual decrease in pRb expression. The pRb immunoreactivity in SCC is frequently lower than the overall CIN cases. The down regulation of retinoblastoma (RB) protein may indicate mutation of RB gene in cervical lesions and it could be involved in cervical carcinogenesis.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest

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