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International Journal of Current Research
Vol. 7, Issue, 11, pp.22657-22660, November, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EPITHELIAL DYSPLASIAS AND THEIR HORMONE RECEPTOR EXPRESSIONS IN HUMAN UTERINE CERVIX CAUSED BY HUMAN PAPILLOMA VIRUS

***,[1]Madasamy Balamurugan, [2]Anand Shankar RaoPatil, [3]Baskar Krishnan and [4]Rashmi Vyas**

[1]Department of Pathology, Tagore Medical College and Hospital, Tamilnadu Dr.MGR Medical University, Chennai – 600127, Tamilnadu, India
[2]Department of Pathology, Sri ManakulaVinayagar Medical College and Hospital, Pondicherry University, Puducherry – 605107
[3]Department of Biochemistry, Sir Raja Muthiah Medical College and Hospital, Annamalai University, Chidhambaram, Tamilnadu – 608001, India
[4]Pondicherry University, Pondicherry – 605107, India

ARTICLE INFO

Article History:
Received 03rd August, 2015
Received in revised form
09th September, 2015
Accepted 26th October, 2015
Published online 30th November, 2015

Key words:

Human Papilloma Virus,
Oestrogen receptor, Progesterone receptor,
Low Grade Squamous Intraepithelial Lesion (LSIL), High Grade Squamous Intraepithelial Lesion (HSIL).

ABSTRACT

Caner cervix is a major cause of morbidity and mortality in developing countries like India. The pre-neoplastic stages of cancer cervix are Low Grade Squamous Intraepithelial Lesion (LSIL) and High Grade Squamous Intraepithelial Lesion (HSIL). The major aetiology is high-risk human papilloma virus infection by subtypes like HPV 16,18,33 etc. The human uterine cervical epithelium responds to the sex hormones like oestrogen and progesterone. Many neoplastic and pre-neoplastic conditions of the cervix are known to alter the expression of these receptors. This study aims to evaluate the various degrees of expression of oestrogen and progesterone receptors in epithelial dysplasias of human uterine cervix caused by High Risk Human Papilloma Virus. 80 cases of cervical biopsies were subjected to Polymerase Chain reaction for High Risk Human Papilloma Virus and histological grading. Immunohistochemical staining was done and nuclear positivity for oestrogen and progesterone receptors was assessed, tabulated and statistically analysed. Both receptors showed significant reduction in the expression of ER and PR in increasing grade of epithelial dysplasia and absent in carcinoma. Dysplastic and malignant changes in the epithelia are accompanied by down regulation of the hormone receptors. This might help in the early detection of cervical cancer.

***Citation*:** Madasamy Balamurugan, Anand Shankar RaoPatil, Baskar Krishnan and Rashmi Vyas, 2015. "Epithelial Dysplasias and their hormone receptor expressions in human uterine cervix caused by human Papilloma virus", *International Journal of Current Research*, 7, (11), 22657-22660.

INTRODUCTION

Caner cervix can be effectively prevented. Many developed countries have achieved near eradication of this cancer. This is due to the fact that it progresses gradually and easily accessible for examination and screening by PAP smear. The pre-neoplastic stages of cancer cervix are Low Grade Squamous Intraepithelial Lesion (LSIL) and High Grade Squamous Intraepithelial Lesion (HSIL) leading to Squamous Cell Carcinoma (SCC). Still it is a major cause of morbidity and mortality in many developing countries like India. There are many etiological factors for cervical cancer which includes smoking, oral contraceptive use, immune suppression and hormone replacement therapy (Angiolo *et al*., 2011).

***Corresponding author: Madasamy Balamurugan**
Department of Pathology, Tagore Medical College and Hospital, Tamilnadu Dr.MGR Medical University, Chennai – 600127, Tamilnadu, India

But the major aetiology is high-risk human papilloma virus (HR-HPV) infection by subtypes like HPV 16, 18, 33 etc. The human uterine cervical epithelium responds to the sex hormones like oestrogen and progesterone. This is due to the presence of corresponding Oestrogen Receptor (ER) and Progesterone Receptor (PR) on the nuclear membrane (Remoue *et al*., 2003). The growth and differentiation of the epithelia is regulated by the hormones via these receptors (Sarpin *et al*., 2009).

These receptors belong to the nuclear receptor super family of transcription factors. Oestrogen receptor-α is the major receptor in the uterine cervix (Pearce *et al*., 2004). Many neoplastic and pre-neoplastic conditions of the cervix are known to alter the expression of these receptors (Chung *et al*., 2013).

MATERIALS AND METHODS

Study subjects

Inclusion criteria: Women aged 30-60 years were selected. Exclusion criteria: History of hysterectomy/ conisation/ previous treatment of cervical cancer. This study was approved by the Institutional Human Ethical Committee (IHEC).

Sampling

Punch biopsy of the cervix was taken. It was divided in to two. One part was stored in Phosphate Buffered Saline (PBS) for PCR. The other part was preserved in Neutral Buffered Formalin (NBF) 10% for histopathological studies.

Screening for HPV

The PBS stored cervical tissue sample was used for PCR. HR-HPV was detected using (*AmpliGenei HPV detection kit, Bangalore Genei, India*). The samples which were confirmed for HR-HPV by type-specific PCR (HPV 16, 18, 31, 33, 35, 45, 52 and 58) were separated. The negative cases were used as controls. The total number of patients thus selected was 80.

Histological grading

The cervical biopsies stored in the formalin were processed by the routine histopathology method to obtain paraffin blocks. The sections were stained by Hematoxylin & Eosin method and evaluated microscopically. They were graded to normal, LSIL, HSIL and SCC. Apart from the 20 normal biopsies which acted as controls for hormone receptor estimation there were 20cases of LSIL, HSIL and SCC.

Immunohistochemistry for ER & PR

Immunohistochemistry was done from the paraffin sections using DAKO IHC kit by Labelled Streptavidin-biotin (LSAB) method. For Oestrogen receptor *DAKO Monoclonal rabbit Anti-Human Oestrogen receptor-α antibody clone SP1* and for Progesterone receptor *DAKO Monoclonal mouse Anti-Human Progesterone receptor antibody clone PgR636* were used. The nuclear positivity of both receptors was assessed and the results were tabulated. The results were analysed by Chi-Squared test.

RESULTS

Out of the 80 biopsies selected which include normal, LSIL, HSIL and SCC, the expression of ER and PR was estimated (Figures 2 to 9) and tabulated (Tables 1 & 2).

Table 1. Immunohistochemical expression of Oestrogen Receptor (ER)

Study population (n=80)	Number of positive biopsies for Oestrogen Receptor	Percentage %
Controls (n=20)	17	85
LSIL (n=20)	5	25
HSIL (n=20)	2	10
SCC (n=20)	-	-

Data are expressed as percentage. (Each category n=20).
Chi-squared test: Calculated value 26.12 vs Table value 5.99.

Table 2. Immunohistochemical expression of Progesterone Receptor

Study population (n=80)	Number of positive biopsies for Progesterone receptor	Percentage %
Controls (n=20)	15	75
LSIL (n=20)	8	40
HSIL (n=20)	5	25
SCC (n=20)	-	-

Data are expressed as percentage. (Each category n=20).
Chi-squared test: Calculated value 15.66 vs Table value 5.99).

The loss of receptors in increasing dysplasias can be appreciated in the comparative bar chart (Figure 1).

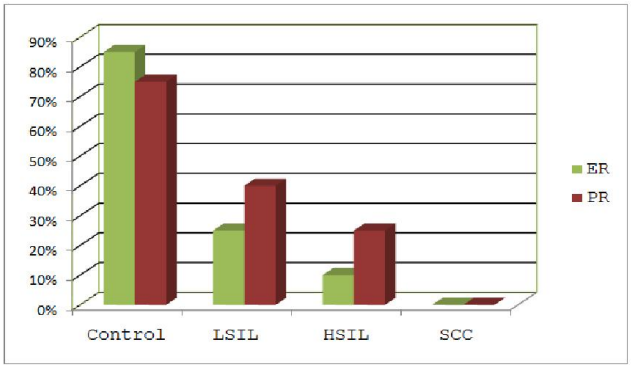

Figure 1. Comparative expression of ER and PR in dysplasias of cervix including SCC

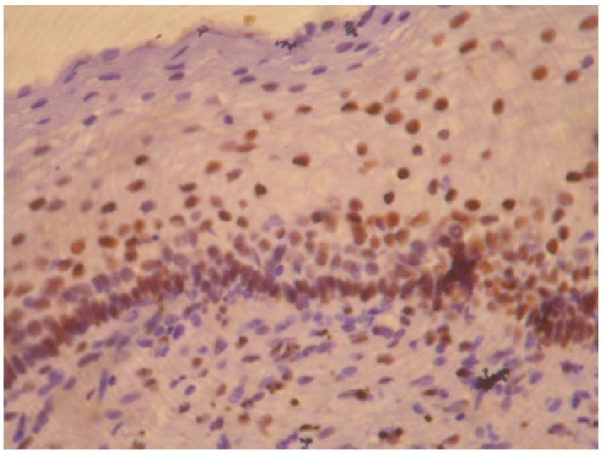

Figure 2. ER expression in normal cervix

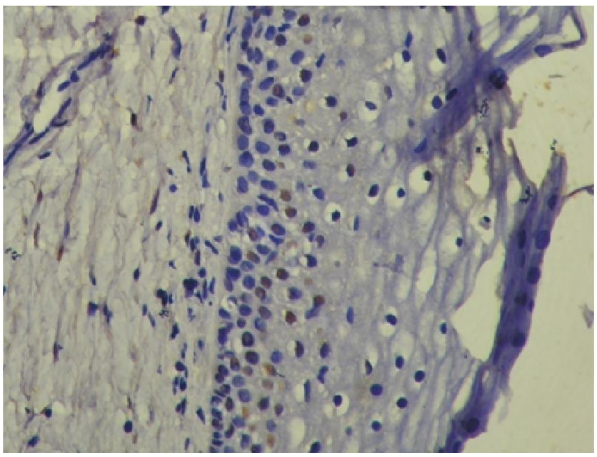

Figure 3. ER expression in LSIL

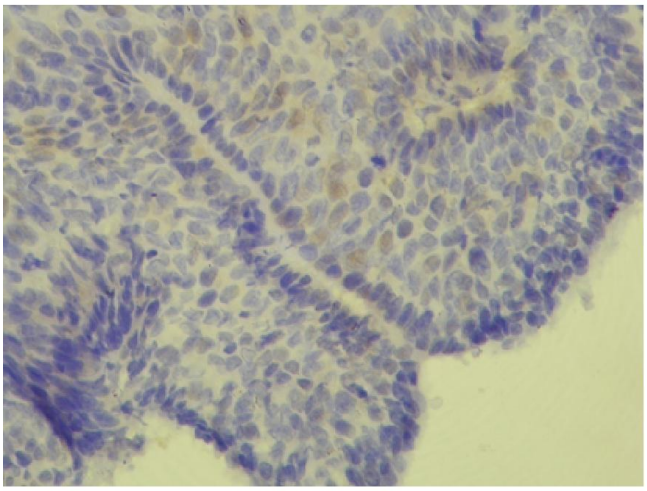

Figure 4. ER expression in HSIL

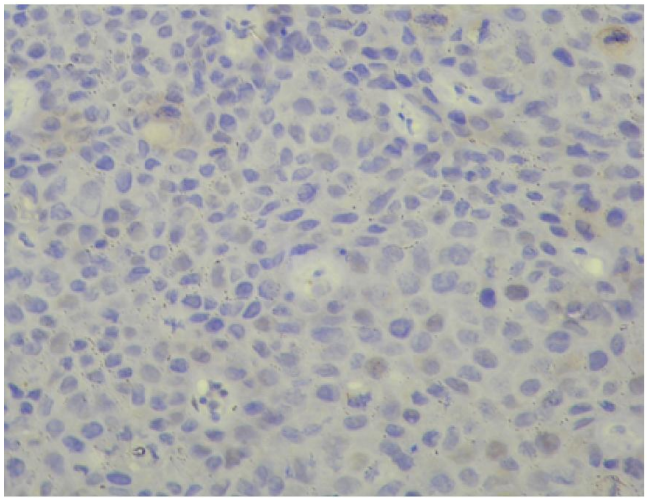

Figure 5. ER expression in SCC

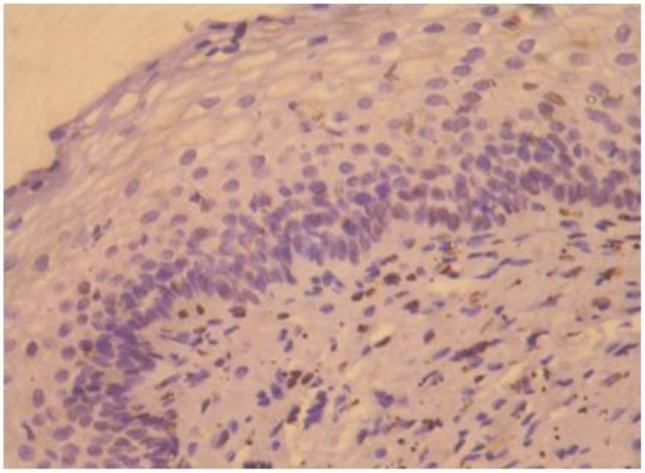

Figure 6. PR expression in normal cervix

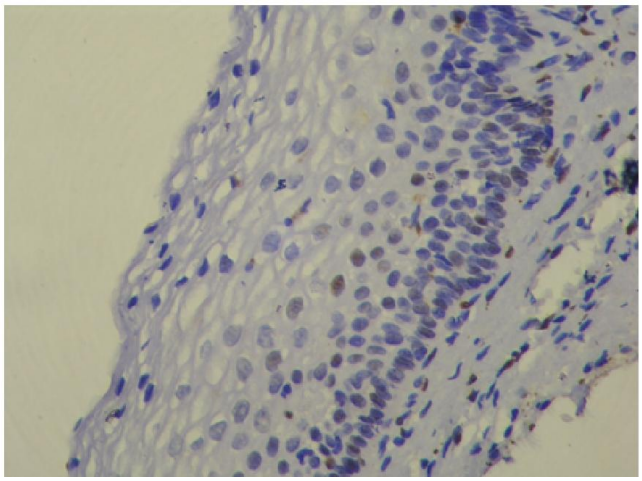

Figure 7. PR expression in LSIL

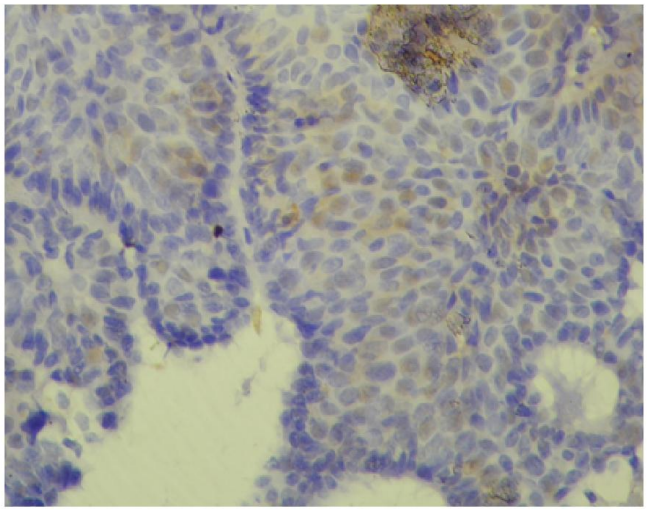

Figure 8. PR expression in HSIL

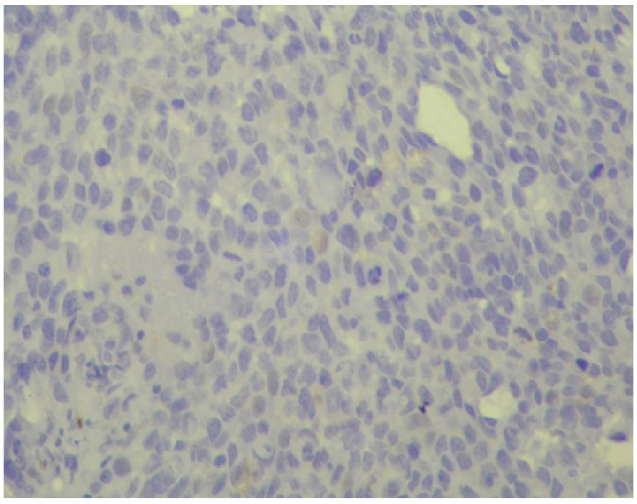

Figure 9. PR expression in SCC

DISCUSSION

Down regulation of ER expression may be theearly alteration to take place in normal epithelium during the development of cervical dysplasia in women infected with HR-HPV (Chung *et al*., 2010). This indicates a loss of normal growth control by sex steroid hormones (Bekkers *et al*., 2005). Studies in animal model showed that the ER required for the early stage of

cervical carcinogenesis. It suggests that ER is no longer required once the critical precursor lesion developed (Chung *et al*., 2008) Transcriptional repression by other transcription factors is likely to contribute to loss of ER during cervical cancer progression (Zhai *et al*., 2010). PR assembly is an oestrogen-dependent process; the presence of functional ER may be necessary for the formation of PR (Coelho *et al*., 2004). Our data indicates that there is a loss of ER and PR during the neoplastic transformation. The loss of ER expression was more prominent than that of PR. Loss of HR expression may be associated with malignant transformation in the HR-HPV infected cervical epithelium. Our result can be compared to Kanai *et al*. (1998) who reported ER and PR expression in normal cervical epithelia and marked reduction of ER and moderate reduction of PR in neoplastic lesions. Our results are contradictory to Kim *et al.* (1992) who reported increased expression of ER and PR in SCC. In spite of contradictory results in some studies, our study showed a consistent decrease in the expression of HR with increasing degree of dysplasia. This decline in the expression of the receptors may help in early detection and prevention of cervical cancer. Also, the detection of these hormones oestrogen and progesterone in the blood may be a prospective study for early cancer detection. Various other markers like potassium channels, human papilloma virus L1 capsid protein, P-glycoprotein, matrix metalloproteinase 2 and p16(INK4a) are also being investigated as an early marker of cervical cancer (Oritz *et al*., 2011,Lee, Sung, 2011, Singh *et al*., 2012, Lee HJ, Kim JW, 2013, Nankivell *et al*., 2014). Also traditional Indian herbal molecules like curcumin are studied for their role in suppressing estradiol and reduce the incidence of cervical cancer (Mayank Singh, Neeta Singh, 2011).

Acknowledgement

We acknowledge the support received from the Department of Biochemistry, Sir Raja Muthiah Medical College and Hospital, Annamalai University, Chidhambaram, Tamilnadu – 608001, India.

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