Estrogen and Progesterone receptor expression in vulvar Condyloma acuminata

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Abstract

Background & objectives: Human papilloma virus is one of the most common sexually transmitted diseases which have different clinical appearances such as Condyloma acuminate (CA) .Its prevalence is more common in women with preponderance in pregnancy which can be attributed to especial hormones such as estrogen and progesterone. The aim of this study was to evaluate the estrogen and progesterone receptor expression in CA lesions of vulva.

Material and Methods: Patients referred to outpatient clinic of Emam Reza hospital suspected of having CA lesions from September 2009- March 2011 were evaluated. Biopsy specimens from CA lesions were taken from 39 patient. Tissue samples was collected and after pathologist confirmation, immunostaining was done on cryostat sections by the peroxidase –antiperoxidase method to measure progesterone and estrogen receptors.

Results: From 37 suspected specimens 25 samples which were confirmed by pathologist To be condyloma acuminata evaluated by IHC;PR was positive in 77,8% of all contaminated samples and 15.4% in normal tissue (p=.000).ER was positive in 33.3% and 25% in contaminated and normal samples respectively**.**

Conclusion: According to the results of this study there is a significant difference in PR receptor expression in CA of vulva.

Keywords: Condyloma acuminate, estrogen receptor, progesterone receptor, Immunohistochemistry, Vulva

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Introduction

Human papilloma virus (HPV) is one of the most common sexually transmitted diseases with a prevalence rate of 24%to 60% in DNA detected studies (1, 2, 3).

Some studies have shown that HPV infections are influenced by sex steroid hormones especially in cervical neoplasias (4) via an alteration of viral genes or by acting as a cofactor in these lesions (4).

Some authors detecting estrogen receptor (ER) in condyloma acuminata (CA) by ImmunoHistoChemistry (IHC), found that positive ER was present in most of CA cases while a few in normal tissues. This shows ER positivity indicative of hormonal dependence (5).Most studies have focused hormonal receptor expression in cervical dysplasia and neoplasias but the lack of evidence exists in CA lesions of vulva.

The question exists why there is a preponderance of CA lesions in females and in pregnancy which imposes the question of CA pathogenesis and the probable hormonal effects in its pathogenesis. The aim of our study was to evaluate the association between sex steroid receptors (ER and PR) expression in normal epithelium of vulva in comparison to CA lesions due to lack of information in hormonal pathogenesis of vulvar lesions.

Methods and Materials;

The work was carried on outpatients of gynecologic clinic with the complaint of genital wart from 2009-2011, after survey confirmation by Kermanshah University Research and ethics

Committee. The age of women was between 18-69 years with median of 33.3+/-13.33 year old. After visual confirmation 37 suspected vulvar CA specimens were collected, informed consent was taken from all cases and a biopsy from the lesion was undertaken by a scalpel number 21 not to destroy tissues. The tissue was divided in to equal parts to contain normal and pathologic lesion, then fixed by buffered formalin solution and transported to laboratory for tissue confirmation and IHC assay of ER and PR receptors. From 37 women suspected of having genital warts, 25 specimens was approved by pathologist, the remaining 12 were used as control group for ER and PR staining.

All tissues were evaluated by H&E stain and pathologic diagnosis of CA was confirmed by a pathologist using published criteria. Immunostainig for ER and PR was done on cryostat sections by peroxidase- antiperoxidase (PAP) method using the ER ICA monoclonal kit(Abbot ,North Chicago , IL)in brief 4um cryostat sections were mounted on glass slide ,coated with the tissue adhesive provided in the kit and placed in 3.7% formaldehyde in phosphate –buffered saline (PBS) for 10 minutes.Sections were treated with 0.3% hydrogen peroxide diluted in methanol to block endogenous peroxidase activity and incubated with normal goat serum.

Then the slides incubated with antiestrptrophilin monoclonal antibody(H222) or control rat immunoglobulimne (Ig) G for 30 minutes at room temperature, followed by treatment with goat anti-rat IgG antiserum(bridging antibody) and with PAP complex. Finally, diaminobenzidine and 0.06% hydrogen peroxide diluted in PBS were applied .Counter staining was done with hematoxylin or methyl green. The specific staining was observed as brown –colored granules, and the control slides as treated with control antibody yielded negative results (Fig -1)Histologic diagnosis of the specimens was made using both the routinely processed hematoxyline eosine –stained sections(Figure -2).

Statistical Analysis: By using SPSS software, the ER and PR status (positive Vs negative) in CA and normal tissue were assessed, using the chi-square test and a probability level of < 0.05 was considered to represent statistical significance.

Results:

Immunohistochemical localization of ER

Normal vulvar epithelium in control group; Anti-ER receptor antibody was confined to the nucleus .Three (25%) out of 12 control specimens were positive for ER in contrast to 9 (75%) negative for ER .

Vulvar CA lesions; In the case of CA of vulva ER positivity was documented in 8/25 of case group (32%) while negative in 17/25 (68%) with no significant difference in estrogen receptor expression in normal and CA affected tissues.

Immunohistochemical localization of PR

Normal vulvar epithelium; in 2 out of 12 normal tissues (16.67%) of controls, PR was positive.

Vulvar CA lesions; in 19 of 25 cases of affected CA tissues progesterone receptor was positive 76% (P =0.005) .A clear contrast was documented between PR expression in CA affected tissues in contrast to controls (Table 1).

By excluding pregnant patients from consideration the same results were obtained in PR and ER expression with significant difference in PR and non significant in ER (Table 2)

Table1 =ER and PR expression in patients with CA lesions.

	ER+	**ER-**	**PR+**	**PR-**
CA+(25)	**8(32%)**	**17(68%)**	**19(76%)**	**6(24%)**
CA-(12)	**3(25%)**	**9(75)**	**2(15.4%)**	**10(84.6%)**

Table 2=ER and PR expression in nonpregnant patients in CA lesions.

	ER+	**ER-**	**PR+**	**PR-**
CA+(22)	**7(32%)**	**15(68%)**	**17(78%)**	**5(23%)**
CA-(12)	**3(25%)**	**9(75)**	**2(16%)**	**10(84%)**

Discussion:

The results of our study showed that in cases of pathologic proven CA the majority of lesions (76%) had PR expression and a minority (24%) were negative while normal control tissue showed 16.67% PR positive in contrast to 83.33% negative with a significant difference .These results about ER in CA were 68% negative and 32% positive while in normal control tissue 75% negative and 25% positive with no significant difference.

Human Papilloma Virus and Herpes simplex virus are the most common genital viral infections in clinical practice worldwide (2).

The typical exophytic CA is recognized by acanthosis, papillomatosis, hyperkeratosis, and viral cytopathic effects (koilocytosis) in the upper epithelial cell layer. (6)Vulvar lesions such as CA, flat condyloma ,classic vulvar intraepithelial lesions (Bowen's disease) and 40% of invasive squamous cancers are attributed to HPV infections.(6).Vulvar CA are not precancerous but

considered one of STIs(6). Two clinical syndromes are attributed to HPV infection; benign anogenital wart and anogenital dysplasias that may progress to cancer (7).

Low risk HPV or subtype 6, 11 cause benign lesions such as genital warts which are very infectious cases (7). These viruses infect the stem cells of basal layer of genital epithelium and their life cycle is associated with keratocytes only in the most differentiated superficial cells (8).The etiology of the viral affinity to genital epithelium is unclear, one hypothesis would be the hormonal milieu of especial cells which attracts viral attacks, this may explain the high prevalence of CA in females in comparison to males. This theory has been studied in cervical tissues to explain the expression of ER and HPV DNA in reproductive tissue of uterine cervix (9). Diagnosis is based on gross appearance however laboratory techniques such as PCR and dot blot have been undertaken (10).By using IHC more information is gathered to best clinical management (11).

ER and PR role in breast and endometrial cancer are well recognized (12) .ER and PR expression have been shown in 34-56% of cervical carcinomas and PR in 15-58% with unknown prognostic role (12). Another study suggests that in cervical malignancies ER loss may be accompanying factor in pathogenesis (13).

In a survey by Hareesh et al increased aromatase activity along with increased estrogen receptor concentration and decreased progestron receptor in cervical cancer in contrast to precancerous lesions suggested estrogen role in cancer pathogenesis (14).In a study by Cui MH et al, ER in CA tissue was found by IHC in most of lesions and in a few of normal vaginal and cervical tissues (5) which is in contrast to our findings.

It's suggested by some authors that PR acts as a tumor suppressor in cervix which is also true in ovarian cancer, many mechanisms have been theorized (14).The increased level of estrogen and cell proliferation in cervical tumor suggests that aromatase is triggered by estrogen biosynthesis and leads to tumor growth (14) .A preponderance of PR may be implicated as a cofactor in cervical neoplasia which can partially explain the malignant transformation of cervix in contrast to vulva (4). Many reports have been published on the ER and PR expression in cervical cancer induced by HPV but scarce information still exists in its expression in vulvar HPV lesions and CA (9, 15).

Normal vulvar tissue and different lesions of vulva have been surveyed for hormonal milieu receptors such as vulvar cancers, Cavernous lymphangiomas, angiomyofibroblastoma, angiomyxoma, indicating ER and PR importance in disease pathogenesis (16, 17, 18, 19) the positivity rate for ER in normal vulvar tissue varies from 33-100% (20) and it's also positive in most of CA lesions and normal vaginal epithelium (5).which was confirmed by our study. On

the other hand studies on PR expression has shown that transformation of cultured cells by HPV DNA 16-18 is primarily under the existence of progestron or progestins of oral contraceptives(21) so many HPV infections may be associated with PR positivity which is in accordance to our finding.

Established ER presence allows the consideration of the introitus of the vagina as a target for estrogen therapy in various clinical and surgical situations. Continuing elucidation of the immunohistochemistry of this external genital tissue might assist in the development of molecular tools to treat genital abnormalities (22).

Conclusion;

These results suggest that hormonal milieu acts as a promoting environment for viral elements to develop ,the exact mechanism should be evaluated whether it's the ER or PR presence which facilitates viral infection or on the contrary it's the viral affinity which changes hormonal status, more information are required.

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Figure 1 .

Figure 2