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Association of estrogen and progesterone with cancer of the uterine cervix in women infected with high-risk human papillomavirus

Krishnan Baskaran[1],*, Kranthi Kumar P[2], Santha K[1] and Inmozhi Sivakamasundari[1]

[1]Department of Biochemistry, Rajah Muthiah Medical College & Hospital, Annamalai University, Annamalainagar, Tamil Nadu, India
[2]Department of Genetics, Narayana Medical College & Hospital, Nellore, Andhra Pradesh, India

Abstract

Background: High-risk human papillomavirus (HR-HPV) infection is essential for the development of dysplasia and cervical cancer. Steroid hormones are implicated as risk factors for cervical carcinogenesis. Thus the aim of the present study is to investigate the association of serum levels of estrogen and progesterone with cervical cancer in HR-HPV infected women.

Methods and materials: The present study consisted of 103 subjects infected with HR-HPV from low-grade squamous intraepithelial lesion (LSIL) to cervical cancer. They included 37 premenopausal women (luteal phase) and 43 postmenopausal women as cancer cases (high-grade squamous intraepithelial lesion and cervical cancer). Twelve women with LSIL for premenopausal and 11 women with LSIL for postmenopausal were chosen as controls. The concentration of estradiol and progesterone were estimated using enzyme linked immuno sorbent assay kit. The prevalence of HPV infection was expressed as percentage of HPV positives. The data for estradiol and progesterone were expressed as mean ± SD.

Results: The serum levels of estradiol were not significantly altered in premenopausal and postmenopausal cases ($p > 0.05$). However, the serum levels of progesterone were significantly increased in premenopausal cases as compared to premenopausal controls ($p < 0.025$). The serum levels of progesterone were not significantly altered in postmenopausal cases ($p > 0.05$). The ratio of estradiol to progesterone was significantly decreased in premenopausal cases ($p < 0.001$) where as it was not significant in postmenopausal cases ($p > 0.05$).

Conclusion: A significantly elevated levels of progesterone is associated with cervical cancer in premenopausal women infected with HR-HPV.

Keywords: Cervical dysplasia; cervical cancer; steroid hormones; estrogen; progesterone

Introduction

Cervical cancer is the fourth most common cancer affecting women and seventh overall worldwide [1]. About 70% of the global burden falls in developing countries and more than one fifth of all new cases are diagnosed in India. For instance, in India, it is the most common cancer among women with more than 130,000 new cases and 70,000 deaths occurring every year [2]. Cervical cancer is usually preceded by a long pre invasive phase, known as dysplasia. Cervical dysplasia is referred histologically as cervical intraepithelial neoplasia (CIN) or cytologically as squamous intraepithelial lesion (SIL) [3]. Specific genotypes of human papillomavirus (HPV) are the single most etiological agents of cervical intraepithelial lesions and cervical cancer [4, 5].

Infection and persistence of high-risk HPV (HR-HPV) are essential for the development of dysplasia and cervical cancer [5]. HR-HPV infections are very common among sexually active young women. Most women clear the infection within one to two years and only a certain women develop dysplasia which later progresses to cervical cancer [6]. Therefore, it implies that HR-HPV infection itself is inadequate and many other factors might play a role in the development of dysplasia and progression to cervical cancer [7]. For example, steroid hormones are implicated in the development of pre neoplastic lesions of the uterine cervix [8].

Prolonged use of oral contraceptive pills (OCP) containing either combined estrogen - progesterone or progesterone alone is associated with an increased risk of cervical cancer in women infected with HR-HPV [9-12]. Chronic estrogen treatment of HPV 16 transgenic mice developed dysplasia [13] and neoplasia in the cervix [14, 15]. These studies clearly indicate estrogen as carcinogen in uterine cervix and epidemiologic studies show that estrogen as carcinogen in endometrium and breast [16]. However, serum levels of these endogenous hormones in HPV infected women have produced inconclusive and inconsistent results [17, 18]. Therefore, the present study was undertaken to investigate the association of serum levels of estrogen and progesterone with cervical cancer in HR-HPV infected women from a hospital-based study.

Materials and methods

A total of 103 subjects within the age group of 30 to 65 years with positive for the HR-HPV infection by polymerase chain reaction (PCR) were selected for the present study in outpatient clinic in the Department of Obstetrics and Gynecology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamil Nadu, India and in the Department of Obstetrics and Gynecology, Thanjavur Medical College, Thanjavur, Tamil Nadu, India, from August 2008 to July 2010. The study subjects belonged to the rural population of Cuddalore and Thanjavur districts of Tamil Nadu, India. Subjects with history of hysterectomy/ conization/ previous treatment of cervical cancer, and currently undergoing any treatment for cervical diseases, presently pregnant, suffering from any serious and systemic illness like cardiac disease, any other malignancy and other sexually transmitted diseases were excluded. The study was approved by the Institutional Human Ethics Committee (IHEC) and the informed consent was obtained from each subject.

Blood samples obtained by venipuncture in serum separating tube (SST) vacutainer (BD, Franklin Lanes, NJ, USA) were allowed to clot and the serum was separated by centrifuging at 3000 rpm for 15 min and used for hormone assay. Cervical scrapings were collected using a sterile disposable cervical brush (Astra Scientific Systems Pvt. Ltd. Kerala, India) in a sample collection buffer [Phosphate buffer saline (PBS) pH 7.4] for the detection of HPV DNA. Biopsy specimens were obtained using a punch biopsy in neutral-buffered formalin [(NBF) (10%)] for histopathological studies. The histopathological grading was performed according to World Health Organization (WHO) classification [19]. There were a total of 63 subjects with cervical cancer. They consisted of 61 squamous cell carcinoma (SCC) and two adenosquamous carcinoma (ADSC). Out of these, 58 subjects with SCC were included in the study. SIL was classified according to Bethesda System 2001 [20]. There were a total of 45 subjects with SIL: 22 subjects with high-grade squamous intraepithelial lesion (HSIL) while 23 with low-grade squamous intraepithelial lesion (LSIL).

Amplification of HPV DNA

Detection of HPV infection with genotyping was performed by PCR (ApmliGenei HPV detection kit, Bangalore Genei, Bangalore, India). This kit detects eight HR-HPV types: HPV 16, 18, 31, 33, 35, 45, 52 and 58. These eight HR-HPV types are responsible

for about 90% of all cervical cancers worldwide [7]. These sub types were detected based on the PCR product size varying between 230 and 270 bp (base pair). The detailed findings of these sub types and their associations with cervical cancer were earlier published by us [21].

Study population

Out of 103 subjects infected with HR-HPV from LSIL to cervical cancer; the study population was segregated for hormone assay according to their menstrual status. They included 37 premenopausal women (from HSIL and cervical cancer as cases) matched according to their menstrual status (luteal phase) and 43 postmenopausal women (from HSIL and cervical cancer as cases) matched years since menopause and age. Twelve women with LSIL were chosen as controls for premenopausal and 11 women with LSIL were chosen as controls for postmenopausal. This was adopted from the earlier publication by Shields et al. [17].

Estimation of hormones

The concentration of estradiol (17-β estradiol) was estimated using enzyme linked immuno sorbent assay (ELISA) kit (E2 – EAISA, Biosource Europe, S.A., Nivelles, Belgium). The assay was performed in duplicates as per the manufacture's instructions. Briefly, 50µl of each calibrator, control and sample was dispensed into the appropriate wells in the micro titer plate followed by 50 µl of estradiol-HRP conjugate and 50µl of anti-estradiol. The plate was incubated for 2 hrs at room temperature on a horizontal shaker set at 700 RPM. The contents were aspirated from the wells, and then washed with 0.4 ml of wash solution for a total of five washes. 200µl of freshly prepared substrate solution was added. The plate was incubated for 30 min at room temperature on a horizontal shaker set at 700 RPM. 50µl of stop reagent was added and the absorbance of each well was read at 450 nm. The values are expressed as pg/ml.

The concentration of progesterone was estimated using ELISA kit. The assay was performed in duplicates as per the manufacture's instructions. Briefly, 50µl of each standard, control and sample was dispensed into the appropriate wells in the micro titer plate followed by 200 µl of progesterone - HRP conjugate. The plate was incubated for 3 hrs at room temperature on a horizontal shaker set at 700 RPM.

The contents were aspirated from the wells, and then washed with 0.4 ml of wash solution for a total of three washes. 200µl of freshly prepared substrate solution was added. The plate was incubated for 30 min at room temperature on a horizontal shaker set at 700 RPM. 50µl of stop reagent was added and the absorbance of each well was read at 450 nm. The values are expressed as ng/ml.

Statistical analysis

The data for estradiol and progesterone were expressed as mean ± SD. Statistical analysis was performed using a Student's *t* test. The results were considered statistically significant if the *p* values were 0.05 or less.

Results

Table 1 shows the serum levels of estradiol and progesterone in premenopausal controls and cases. The serum levels of estradiol were not significantly altered in premenopausal cases as compared to premenopausal controls (p>0.05). However, the serum levels of progesterone were significantly increased in premenopausal cases as compared to premenopausal controls (p<0.025).

Table 1: Serum estradiol and progesterone levels in premenopausal women.

Hormones	Controls	Cases	P value
Estradiol (pg/mg)	71.28 ± 12.48	76.16 ± 14.65	NS
Progesterone (ng/ml)	1.75± 0.29	2.39 ± 0.94	< 0.025

Note: Data are expressed as mean ± SD. Control (n=12); Cases (n=37); NS: Not significant.

Table 2 shows the serum levels of estradiol and progesterone in postmenopausal controls and cases. The serum levels of estradiol and progesterone were not significantly altered in postmenopausal cases as compared to postmenopausal controls (p>0.05). Table 3 shows the estradiol/ progesterone ratio in premenopausal controls and cases. The ratio of estradiol to progesterone was significantly decreased in premenopausal cases as compared to premenopausal controls (p<0.001). Table 4 shows the estradiol / progesterone ratio in postmenopausal controls and cases. The estradiol to progesterone ratio

was not significantly altered in postmenopausal cases as compared to postmenopausal controls (p>0.05).

Table 2: Serum estradiol and progesterone levels in postmenopausal women.

Hormones	Controls	Cases	P value
Estradiol (pg/mg)	39.17 ± 8.64	42.57± 7.51	NS
Progesterone (ng/ml)	1.18 ± 0.19	1.71 ± 0.86	NS

Note: Data are expressed as mean ± SD. Control (n=11); Cases (n=43); NS: Not significant.

Table 3: Serum estradiol/ progesterone ratio in premenopausal women.

Parameter	Controls	Cases	P value
Estradiol/ progesterone ratio	44.67 ± 6.43	37.52 ± 8.14	<0.001

Note: Data are expressed as mean ± SD. Control (n=12); Cases (n=37).

Table 4: Serum estradiol/ progesterone ratio in postmenopausal women.

Parameter	Controls	Cases	P value
Estradiol/ progesterone ratio	31.74 ± 8.54	36.24 ± 11.6	NS

Note: Data are expressed as mean ± SD; Control (n=11); Cases (n=43); NS: Not significant.

Discussion

Estrogens are a group of steroid hormones responsible for the primary and secondary sexual characteristics in females [22]. Many experimental molecular studies produced results in support of estrogens as carcinogen in cervical cancer; however, there has been inconsistency in the results of studies done on association of serum estrogen levels with cervical cancer. One epidemiological study reported the association of serum estrogen levels with cervical cancer [23]. However, Shields et al. [17] in a case-control study showed no significant association of serum estrogen levels with cervical cancer. Wang et al. [18] in a case-control study reported that there was an association of serum estrogen levels with cervical cancer. The present study did not provide a significant association of serum estradiol with premenopausal and postmenopausal cancer cases.

Progesterone, another steroid hormone, is essential for female reproductive functions that include induction of ovulation, implantation, and maintenance of early pregnancy and stimulation of lobular alveolar development in mammary gland [24, 25]. Though the prolonged use of OCP containing progesterone increases the risk of cervical cancer, it is unclear whether elevated serum levels of progesterone are significantly associated with cervical cancer. Shields et al. [17] reported no clear trends of increasing risk with increasing progesterone levels in premenopausal cases in a case-control study. Wang et al. [18] reported higher levels of progesterone among cases than controls in a case-control study. However, after adjusting with lowest cutoff values in follicular phase, it was not statistically significant in premenopausal cases as compared to controls. The data obtained from our study showed a significant association (p<0.025) of serum progesterone levels with premenopausal cancer cases. This is going with earlier study by Kedzia et al. [26] who reported higher prevalence of HPV infection in women with higher levels of serum progesterone.

As endogenous hormone levels are known to vary throughout a woman's lifetime; the use of a single blood sample to characterize the long term hormonal environment of a woman is a problematic in nature [17]. The ideal study would be prospective, with multiple measures of hormones over time and before detection of invasive disease [17]. Another ideal approach would be assessing the balance between the levels of serum estrogen and progesterone in cervical carcinogenesis. Hence, the present study assessed serum estradiol/ progesterone ratio and found a statistically significant association (p<0.001) between low serum estradiol/ progesterone ratio in premenopausal cancer cases as compared to controls. However, there was no significant difference between serum estradiol/ progesterone ratio in postmenopausal cases and controls. The present study shows that the low serum estradiol/ progesterone ratio has association with cervical cancer among premenopausal women. It is in good agreement with earlier study [27] which reported low estrogen/ progesterone ratio among premenopausal women with cervical cancer. The long control region of HPV contains progesterone response element and progesterone may increase the transcription of E6 and E7, thereby increasing the tendency of cell

transformation [28]. Phase II trial of treatment of vaginal application of progesterone in LSIL showed lower rate of disease regression than controls [29]. In invasive cancer, high serum progesterone levels associated significantly with increased expression of c- MYC and decreased expression of p53 [30]. The present study found an important link between steroid hormone particularly progesterone and cervical cancer in women infected with HR-HPV. However, the limitation is that it is a hospital-based study with convenient sample size. A prospective study with a larger sample size required in order to ascertain the association of progesterone with cervical cancer.

Conclusion

The present study shows a significant association of elevated levels of progesterone with cervical cancer in premenopausal women infected with HR-HPV. However, the significance of this association with HPV positive cervical cancer is not known. A prospective study with a larger sample size and molecular mechanisms underlying the disease progression may lead to further understanding the association of progesterone with cervical cancer.

Conflict of interest

Authors declare no conflicts of interest.

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