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Original Research Article

Clinical significance of HPV-HC2 in diagnosis and prognosis of cervical lesions: a retrospective study

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ABSTRACT

Background: To evaluate the human papillomavirus HC2 different range detection values and their clinical significance in prediction of CIN lesions grades as well as their role in the follow-up outcome after treatment.

Methods: Using the hybrid capture 2 to detect and measure the HPV and the viral load quantity, we enrolled a total of 527 HPV positive women. All patients underwent thin prep liquid-based cytology test (TCT) and only 325 underwent colposcopy guided biopsy due to abnormal cytology results. All cytology and biopsy results were collected and analyzed according to the HPV viral load. Among these patients 108 patients were followed during 2 years post-operatives and their prognosis results were collected and analyzed.

Results: The proportion and severity of cytological abnormalities was positively correlated with the HPV-HC2 viral load ($P < 0.05$). There was a positive correlation between cervical biopsy results and the HPV viral load ($P < 0.05$). The more the HPV-HC2 viral load was, the higher CIN2-3 grade percentage was getting. However no statically significant correlation was found between the HPV-HC2 viral load and the follow up outcomes after treatment ($P > 0.05$).

Conclusions: High HPV-HC2 viral load is significantly associated with the severity of cervical lesions (CIN), however it does not predict any further prognosis on follow-up after treatment.

Keywords: Cervical intraepithelial neoplasia, Follow-up, Human papillomavirus, Hybrid capture 2, Viral load

INTRODUCTION

Human Papillomavirus (HPV) infection is clearly known as the most common cause of cervical intraepithelial neoplasia (CIN) and cervical carcinoma. Generally, the human immune system has the ability to clear up to 90% of HPV infections within 2 years and only 5 to 10% may become persistent and lead to high grade CIN or worse.^{1,2} Factors that lead to persistence and progression from infection to cervical intraepithelial neoplasia and then to cervical cancer include the HPV type and HPV viral load.³

Hybrid capture 2 (HC2) is one of the most commonly used testing methods for identifying the presence of HPV

infection, and at the same time as semi-quantitative detection of HPV virus.

In fact, the relationship between high HPV-HC2 viral load and grade of CIN lesions is still under debate. Several studies have described the degree of cervical lesions as an increasing function with the HPV-HC2 viral load as dependent variable, while in other investigations, no relationship has been reported.^{2,4-7}

Therefore, the purpose of our study is designed to explore the correlation between semi-quantitative detection of HPV virus expressed as relative light units (RLU/CO) obtained by using the Hybrid Capture2 (HC2) assay, with the presence and severity of cervical squamous

intraepithelial lesions, and also to explore the role of HPV viral load in the follow-up monitoring of patients after treatment.

We correlated the HPV viral load detected by HC2 with cytology results, biopsy diagnosis and follow-up results to determine the predictive rule of HPV-HC2 in CIN grade lesions as well as in further prognosis after treatment.

METHODS

Participants and procedures

A prospective study was conducted with women who visited the first affiliated hospital of Nanjing medical university from January 2016 to September 2016 for clinical routine evaluation.

Exclusions criteria were pregnancy, history of hysterectomy, having menstruation or infection during evaluation.

Thin prep smears were conducted along with a collection of cervical cells to determine the status of the cervical epithelium. Colposcopy was suggested in patients with abnormal cytological status, and punch biopsy was done

if any suspicious lesion was identified. Follow-up was carried out during 2 years post-operatives and results were collected according to biopsies underwent within this period.

This research project was approved by the ethics committee of the first affiliated hospital of Nanjing medical university and written informed consent from participants was not necessary as data were extracted from the patient's register of the department of obstetrics and gynecology. All patients were followed at the first affiliated hospital of Nanjing medical university until the biopsy results were provided.

Assessment of viral load

HPV viral load was assessed using HC2 assay according to the manufacturer's instructions (QIAGEN, Inc). In HC2 the DNA: RNA hybrids are captured on a microplate, and the emitted light is measured in a luminometer as relative light unit (RLU/CO. Samples were scored negative with an RLU/CO ratio less than 1, and as positive with an RLU/CO ratio up to 1. Positive cases were identified and a total of 527 patients was recruited and divided into 6 groups according to their HC2 viral ranges. Grouping characteristics are resumed in Table 1.

Table 1: grouping of patients according to their different viral loads.

Subgroups	Group A	Group B	Group C	Group D	Group E	Group F
HC2 (RLU/CO)	1-10	>10-100	>100-200	>200-300	>300-400	>400
Patients	147	108	76	63	29	104
Age*	39.2±9.85	39.1±9.13	40.7±10.2	41.6±10.5	41.2±7.75	40.4±10.2

*P>0.05; 1-way analysis of variance (ANOVA) for patients' age.
HC2 (RLU/CO) = hybrid capture 2(relative light units/cut-off)

Cytological and histological assessment

Cytological evaluation was performed using liquid-based cytology tests, and results were interpreted according to the criteria set by the 2001 Bethesda system for cervicovaginal cytology reposting: NILM for negative results; and ASC-US, LSIL, ASC-H and HSIL for positive results. Women with abnormal cytology results were recalled for colposcopy examinations and punch biopsies were obtained in women who showed any obvious cervical lesions and taken to the pathology department of the first affiliated hospital of Nanjing medical university.

Results were interpreted as chronic inflammation for negative histological results, CIN1 and CIN2-3 for positive histology results.

Follow-up

Follow-up was carried out during 2 years post-operative period in 108 patients who underwent LEEP (Loop Electrosurgical Excision Procedure) treatment for CIN2-3 lesions with different HPV viral load. Medical visits were done once in the first post-operative 3 months and subsequently one time every 6 months using Pap smear and HPV hybrid capture test. Recurrent disease was proved by a colposcopy-directed punch biopsy if any lesions required it. Relationship between the viral load and prognosis after treatment was studied.

Statistical analysis

Statistical analyses were performed using the SPSS software (version 22.0). 1-way analysis of variance

(ANOVA) was used to analyze the age of women in each group.

Total mean age of these woman is 40.0±9.8 and there was no difference in age among groups (P>0.05).

The R.C. chi-square test was used with statistical significance defined as two tailed P<0.05 for all tests.

Relationships between HPV-HC2 load and cytological results

RESULTS

The HPV-HC2 and cytology results are summarized in Table 2.

A total of 527 were enrolled in our study. Table 1 gives an overview of patients' demographic characteristics.

Table 2: Cytology results among patients with different HPV viral load.

Cytological results	Su groups					
	Group A	Group B	Group C	Group D	Group E	Group F
NILM*	101/147(68.7%)	53/108(49.1%)	30/76(39.5%)	20/63(31.7%)	10/29(34.5%)	34/104(32.7%)
ASC-US	42/147(28.6%)	43/108(39.8%)	35/76(46.1%)	30/63(47.6%)	8/29(27.6%)	41/104(39.4%)
LSIL	2/147(1.4%)	6/108(5.6%)	4/76(5.3%)	9/63(14.3%)	8/29(27.6%)	21/104(20.2%)
ASC-H	2/147(1.4%)	4/108(3.7%)	3/76(3.9%)	1/63(1.6%)	2/29(6.9%)	2/104(1.9%)
HSIL*	0(0.0%)	2/108(1.9%)	4/76(5.3%)	3/63(5.1%)	1/29(3.4%)	6/104(5.8%)
total (n)	147	108	76	63	29	104

*P<0.05 chi-square test NILM= negative for intraepithelial lesion or malignancy
 ASCUS= atypical squamous cells of undetermined significance;
 LSIL= low-grade squamous intraepithelial lesion; HSIL= high-grade squamous intraepithelial lesion

Table 3: Relationship between HPV viral load and histology diagnosis.

Histological diagnosis	Sub groups					
	Group A	Group B	Group C	Group D	Group E	Group F
Chronic inflammation	36/53(67.9%)	39/68(57.4%)	18/68(34.0%)	17/44(38.6%)	10/19(52.6%)	35/87(40.2%)
CIN1	8/53(15.1%)	9/68(13.2%)	8/53(15.1%)	6/44(13.6%)	1/19(5.3%)	13/87(14.9%)
CIN2-CIN3*	9/53(17.0%)	20/68(29.4%)	27/53(50.9%)	21/44(47.7%)	8/19(42.1%)	39/87(44.8%)
total	53	68	53	44	19	87

*P<0.05 chi-square test CIN1=cervical intraepithelial neoplasia grade1
 CIN2-3=cervical intraepithelial neoplasia grade2-3

Table 4: Follow-up results.

2 years follow-up results	Sub groups					
	Group A	Group B	Group C	Group D	Group E	Group F
Chronic inflammation	23/22(59.1%)	12/26(46.2%)	4/26(25.0%)	6/12(50.0%)	4/6(66.7%)	15/26(57.7%)
CIN1	2/22(9.1%)	6/26(23.1%)	3/26(18.8%)	4/12(33.3%)	1/6(16.7%)	4/26(15.4%)
CIN2-CIN3*	7/22(31.8%)	8/26(30.8%)	9/26(56.3%)	2/12(16.7%)	1/6(16.7%)	7/26(26.9%)
total n	22	26	16	12	6	26

*P>0.05 chi-square test CIN1=cervical intraepithelial neoplasia grade1
 CIN2-3=cervical intraepithelial neoplasia grade2-3

According to these results, an existing relationship between the HPV quantity and the degree of lesions found in cytological test can be seen.

(28.6%), and ASC-H (1.4%), HSIL (1.4%) was having the lowest percentages compare to other groups.

In group A, majority of patients, 101(68.7%) was observed with normal result NILM and no HSIL lesions was found 0(0.0%). ASC-US rate was slightly high

As HPV-HC2 quantity increase we noted a significant decreasing of NILM percentages in group B;C;D;E;F (49.1%; 39.5%; and 31.7%; 34.5%; and 32.7%), but at the same time a progressively increasing in ASC-US rate

in groups A;B;C;D(28.6%; 39.8%;46.1% and 47.6%), LSIL rate in group A;B;C;D;E;F (1.4%; 5.6%; 5.3%, 14.3%, 27.6%; and 20.2%), ASC-H rate in group A;B;C;E (1.4%; 3.7%; 3.9%; and 6.9%) and also HSIL rate in groups A;B;C;F (0.0%; 1.9%; 5.3%; and 5.8%) was observed. Moreover, analyzing these results using the chi-square test, a P value ($P=0.001$) was found, which confirm a strong evidence of a relationship between the HPV hc2 viral load and the degree of lesion found in cytological test. High HPV-HC2 viral load correlates well with high lesions found in cytology test.

Relationships between HPV-HC2 load and grade of cervical lesions

Among the patients who underwent colposcopy, a total of 325 patients have a punch biopsy done due to unsatisfied results or the presence of low or high cervical lesions. Results are described as: chronic inflammation for normal results, CIN1, and CIN2-3 for abnormal results. As it is shown in table3, high percentage of chronic inflammation or normal result was found in group A patients (67.9%). A decreasing of chronic inflammation rate was observed in group B; C; D; E and F, with HPV-HC2 load increasing: (57.4%; 34.0%; 38.6%; 52.6%; and 40.2% respectively).

A slightly elevated of CIN1 percentages was found in all groups (15.1%; 13.2%; 15.1%; 13.6%; 5.3%; and 14.9% correspondingly to group A, B, C, D, E, F).

However, a significant increasing rate of CIN2-CIN3 lesions was noticed with HPV-HC2 viral load getting higher, starting from 17%; 29.0%; 50.0%; 47.7%; 42.1%; and 44.8% for group A, B, C, D, E, F respectively. Briefly, these results are illustrating a positive correlation between HPV-HC2 viral and grade of cervical lesions ($P=0.011$). High grade cervical lesions occur with a high level of HPV-HC2 viral load.

Follow-up results

Follow-up results are given in Table 4. Among 108 patients with different HPV viral load followed after treatment, rate of good prognosis was found, almost high in all groups with the highest rate found in group A (59.1%) and in group E (66.7%). Recurrence of low-grade lesion (CIN1) was found with slight elevated percentages in all groups (9.1% for group A; 23.1% for group B; 18.8% for group C; 33.3% for group D; 16.7% for group E; and 15.4% for group F). High degree lesion CIN2-3 was found with significant rate in all groups especially in group A, B, C (31.8%; 30.8%; 56.3%). This shows us that, there is no any dependency noticed between the recurrence and rate of low or high grade CIN lesions with the HPV HC2 results. Prognosis of cervical lesions after treatment was self-directed and completely independent to the HPV viral load ($P=0.233$).

Furthermore, combining all our study groups follow-up results together, we have found that of the 108 patients, 54 (50%) have returned to normal, good prognosis, and 54 (50%) have recurrence of CIN lesions. Chance of good prognosis was equal to the chance of redeveloping cervical lesions after treatment and it is not related to the HPV viral load. HPV-HC2 viral load is not a predictive factor of persistence or recurrence of CIN after treatment.

DISCUSSION

HPV infection is the most important factor in the development of CIN and cervical cancer. Relationships between HPV viral loads and the degree of cervical intraepithelial neoplasia become the objective of many investigations and eventually all those studies still lead to controversial results. In this present study, we aimed to re-investigate the role of HPV viral load in cervical lesions grade and also in the follow-up monitoring after treatment.

Firstly, in our study, high rate of abnormal cytology result was found mostly in women with high HPV viral load. LSIL, ASC-H, HSIL percentages was increasing with HPV viral load getting higher. This is to say that high viral load is a factor which can predict some indications about the cytology outcomes and the severity of cervical lesions that can be found with histological test.^{8,9} This can be supported by a cross sectional study done by Flores R et al, which showed high HPV viral load ,especially for oncogenic types is related to the risk of LSIL and HSIL.¹⁰ This is also in accordance with several previous studies, where women with lower viral load was mostly found with NILM as result of cytology test and had lower chance for developing CIN2+, contrary with women with a raised viral load who were mostly found with abnormal cytology results, and had a greater chance of developing CIN2+ lesions or more.^{7,8,11}

Furthermore, in this study, prevalence of CIN2-3 grade lesions was markedly found in women with higher HPV viral load, comparing to those with a lower viral load who were mostly found with normal results or low-grade lesions. High frequency of biopsy-confirmed CIN2-3 was considerably related to elevated viral load detected by the HC2 assay. Similar results have been demonstrated by M. Orioni although in a different setting.¹²

Another research done in ASCUS women showed a low prevalence of CIN 2-3 among women with RLU ratios less than 10 (84.1% had negative results, 11.1% had CIN1, and only 3.2% had CIN2-3).¹³ Possible reasons that can explain the higher prevalence of high grade lesions in high viral load groups may be: chance of clearance of the infection is very diminished in women with higher viral load and that can yield to the persistence of the HPV infection, low regression of cytological abnormalities and then occurrence of cervical lesions or increase severity of the preexisting lesions.⁶ HPV-HC2

value can be used as a predictive tool for cervical lesions severity.

In this current study, we demonstrated that increasing HPV-HC2 viral load correlates significantly with high abnormal cytological status, and mostly with the increasing of CIN2-3 prevalence, a correlation which can be used for improvement of the detection of high-grade cervical lesions.⁹ However, some investigations have shown that this relationship was depending to the HPV type and genotype, and was mostly related to HPV 16 involvement.^{4,14} In addition persistence of high risk HPV infection is considered as an important reason of cervical lesions progressions and HPV 16 is the most known high risk HPV with long persistence infection period.¹⁴⁻¹⁶ Unfortunately, this constitutes a limitation in our studies, as we did not get access to cobas HPV test results which could have allowed us to find the contribution of the HPV types in this relations. Viral load detection by the HC2 assay was shown to be highly sensitive, and the HPV-HC2 viral load could be considerably used as a potential marker for predicting the risk for developing higher cervical intraepithelial neoplasia and cervical cancer.^{14,17}

It has been demonstrated in some studies that patients treated with high grade CIN have a great chance of redeveloping the disease. Borrai F et al, has shown that 5 to 15% of women who underwent treatment for CIN2+ will redevelop the disease within 2 years postoperative.¹¹ According to Jeong NH, 5 to 25% of them will have recurrent disease.¹⁸ In a recent study, rate of persistence of high grade CIN after treatment was 25.7% and it has been shown to be mostly associated with persistent HPV 16 infection.¹⁹ In our study, 50% of patients with different range of HPV viral load, were having persistent CIN lesions, with 31% having CIN2-3. Prognosis was strictly independent to the HPV viral load, and chance of recurrence of cervical lesions (CIN1, CIN2-3) or having better prognosis after treatment was almost the same in all patients whatever how high or low their HPV-HC2 viral load was. This is in contrast with Shen G's investigation which showed that HPV-HC2 viral quantity is an effective indicator for the follow-up and prognosis of cervical lesions after treatment.² Recurrence of the disease could be a result of HPV post-infection but not the present infection and viral load as current lesions were entirely removed during operation with a negative margin status.

However, our results can be reinforced by other studies which showed that factors such as HPV-HC2 viral load, HPV genotype are not predictive for recurrence of disease and the only significant factor that could predict the persistence or recurrence of disease was the LEEP positive margin status.^{20,21}

CONCLUSION

In conclusion to our study, there is an existing strong positive correlation between the HPV viral load detected

by HC2 assay and the severity of cervical lesions. CIN lesions become severe with intermediate to higher HPV viral quantity. HPV-HC2 can be used as a potential indicator for predicting the risk for developing higher cervical lesions in present illness; however, it cannot be used as a predictor for further follow up and prognosis after treatment.

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