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Case series

Colposcopy accuracy in diagnosing cervical precancerous lesions in western Kazakhstan

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ABSTRACT

This retrospective cohort study focused on colposcopic accuracy for the diagnosis of cervical premalignant lesions using cytology and histology, as well as HPV data not included in current cervical screening practices in Kazakhstan. Colposcopy performance was assessed using the modified Reid index in women aged 18–63 years. In total, 1,129 colposcopic-HPV-cytology triple samples and 94 histology findings were collected. The sensitivity of colposcopy was 81.6% with specificity 72.6% for LSIL but fell to 56.6% with specificity 88.3% for CIN2+ vs. 89.6% and 74.5% for cytology at CIN2+, respectively. The ORs for high-grade lesion occurrence within each colposcopy group at viral load rising vs. ORs for HPV-negative women were 3.4; 5.3; and 39.7, respectively ($p < 0.0001$). Total attributive agreement between the colposcopy and histology findings reached 55.3%, κ 0.47 ± 0.06 vs. 0.62 ± 0.08 for cytology, and 0.34 ± 0.13 and 0.58 ± 0.1, for specialists, respectively. Outcomes obtained for colposcopy alone failed to show satisfactory reliability. Globally adopted primary HPV screening would be the best option despite the related costs.

1. Introduction

Cervical cancer (CC) is caused by the group of human papillomaviruses (HPV) for which "there is no perfect way to categorize a continuum of their carcinogenic potential" (Schiffman et al., 2009). In Kazakhstan, a sizeable post-Soviet state in Central Asia, the nationwide CC screening program was implemented in 2008; nevertheless, issues of cancer prevention remain problematic. The screening program includes a Pap test every four years in women aged 30–70 years old using liquid-based cytology (LBC) techniques with "Cell Scan" technology (South Korea manufacturing) and conventional azur-eosin staining as an opportunistic method. In research, the conventional azur-eosin method had a sensitivity of 90.4% and a specificity of 90.0% for CIN2+. Researchers failed to show the LBC "Cell Scan" technique to be superior to simple azur-eosin staining (Balmagambetova et al., 2020). Primary HPV testing accompanied by cytology triage in HPV-positive women aged 30+ has not been adopted in the country, despite the HPV prevalence of approximately 25–28% (Aimagambetova and Azizan, 2018). Reportedly, the CC incidence rate was 18.2 per 100,000 women in Kazakhstan (Bruni et al., 2019).

According to commonly accepted guidelines, colposcopy examination follows screening procedures upon presentation of abnormal cytology (Boardman et al., 2019; NHS Cervical Screening Programme, 2016). Regarding the diagnostic accuracy of colposcopy in various meta-analyses, its sensitivity fluctuated from 29% to 100%, and its specificity ranged from 12% to 88% (Mustafa et al., 2016). At least a rough analysis of colposcopy capabilities for screening purposes would be reasonable given the HPV testing unavailability in the country.

In this study, we assessed colposcopy accuracy for the diagnosis of cervical precancer lesions compared to cytology and histology findings

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by applying HPV data not included in current cervical screening practices in Kazakhstan.

2. Methods

This retrospective cohort study explored the limits of colposcopy within a broad multipurpose research effort on HPV infection across western provinces of the country. The study protocol was approved by the University's IRB and published (Bekmukhambetov et al., 2018). Work was carried out under the Helsinki Declaration principles, and all participants signed the informed consent form. We enrolled women within 18–63 years old and then stratified the sample by age. A total of 1,129 of 1,166 women were asked for their clinical history and were selected for colposcopy. No vaccination history was the only inclusion criterion. HPV vaccination, as well as HIV presence, pregnancy, or any previous procedure on the cervix were exclusion criteria. Cases with verified invasive cancer were not included in the study.

Qualitative detection and quantification of human papillomavirus was performed through real-time PCR based on Russian test systems and equipment. We used the "Quantum-21″ kit for typing and quantifying the DNA of low-risk HPV (6, 11, 44) and high/probable carcinogenic risk (HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) in a total of 21 types. To isolate the viral DNA, sets PROBA-NK-PLUS, the same production, were applied.

We performed colposcopy after PCR-based assays for HPV from the cervix according to standard procedures. To assess cervical condition, we applied the modified Reid colposcopy index (RCI) despite the presence of Swede scores (developed in 2005), because RCI is in use in Kazakhstan. Standard parameters were assessed as presented in the EVAH study (van der Marel et al., 2014), as the objectives and methods applied were similar. The EVAH research focused on studying colposcopic performance in diagnosing high-grade cervical lesions using colposcopic characteristics and high-risk HPV genotyping. Colposcopists identified and graded the lesions and scored the lesions' colposcopic impression, collecting multiple (up to four) biopsies at suspected CIN, including the normal tissue biopsy. Accordingly, we also scored the following parameters: the lesion color, the surface configuration and margins, presence/absence and degree of punctation and mosaicism, vessels, acetowhitening rapidity, and size of the lesions (0, <25, 25–50, >50% of the cervix). Cervical tissue biopsy selectively followed colposcopy examinations at suspected CIN. To systematize findings, we allocated three groups according to a colposcopy opinion: group 1, with scores by RCI up to 2 (supposedly, from NILM to CIN1); group 2, with scores up to 4 (LSIL and overlapping lesion, presumably CIN2); group 3, where scores five and over were referred (HSIL, likely to be CIN3), respectively. Colposcopic terminology adopted in July 2011 was used. The two team members experienced and certified in the cervical pathology were responsible for colposcopy opinion and biopsy sampling. Their experience in colposcopy performance was six and more than ten years, respectively. Cytology findings obtained through the conventional technique (azur-eosin staining) were available for all women and designated according to the TBS (Terminology Bethesda System) 2001, as the research started before the issuing of the new edition of 2016. All histology findings were also combined into three groups and designated group 1 - NILM, up to CIN1; group 2 - LSIL, up to CIN2; and group 3 - HSIL, CIN3.

2.1. Statistical processing:

All calculations were carried out using Statistica 10 (Dell Technologies, Texas, USA). For all tests, a two-sided type I error of $p < 0.05$ at 95% CI was assumed to be statistically significant. Evaluation of colposcopy as a diagnostic tool for the detection of CIN was performed using Cohen's kappa calculation and ROC analysis using SPSS v.25 (IBM, Armonk, NY, USA) and www.medcalc.be. The logistic regression model with OR calculations was designed to evaluate the probability of HSIL development at viral load increments within each RCI group.

3. Results

All colposcopy findings (*n* 1,129) were stratified by HPV status. As such, we performed 846 HPV-negative and 283 HPV-positive assays. Among the HPV-positive women, 60 (21.2%) were low-positive, 96 (33.9%) were moderate, and 127 (44.9%) were of high categories of viral load. Baseline data are provided in Table 1.

A statistically significant age difference, both for HPV-negative and HPV-positive women, has been established when analyzing across the three groups of incrementally increasing changes depending on the colposcopic impressions. The Kruskal-Wallis rank-sum test was $H = 44.47$ (2, $p < 0.05$, *n* 846) and $H = 41.2$ (2, $p < 0.05$, *n* 283). In the meantime, no difference has been found in the mean age of women's sexual debut: $H = 2.93$ (2, p 0.23) for HPV-negative and $H = 1.85$ (2, p 0.39) for HPV-positive sample, respectively.

A noteworthy trend emerged when we assessed cytology findings. In eighty (9.5%) of HPV-negative women having unfavorable colposcopic impressions (RCI scores 5+), high-grade cytology lesions were found. Conversely, almost in a quarter (23.2%) of HPV-positive women with severe colposcopic opinions, the NILM cytology conclusions were obtained. Overall, the number of high-grade lesions in cytology smears (HSIL) was 25 of 1,129 (2.2%): 7 in RCI group-1, 8 in group-2, and 10 in group-3. As to the viral load trends, the proportion of high load (5 + GE*10^3 per sample) was twice as lower in the group-1 (RCI 0–2) compared to the group RCI 5+ (34.7% vs. 68.3%), and the average viral load was expectedly lower (4.7 vs. 7.0). Table 1 also demonstrates a consecutive increase in the share of the most carcinogenic HPV 16/18. The severity of colposcopy changes grows as the proportion of these types rises (Kendall's τ 0.26, p 0.035).

3.1. Assessing the diagnostic value of colposcopy

We performed ROC analysis to estimate colposcopy diagnostic value and assessed the agreement between colposcopists' impressions and histology conclusions. ROC analysis showed that the area under the curve (AUC) was 0.78 ± 0.05 (CI 95% 0.66;0.84) for colposcopy vs. 0.83 ± 0.04 (CI 95% 0.72;0.89) for cytology (p 0.0001).

Table 2 outlines the coordinates of the obtained ROC curve.

The sensitivity of colposcopy with the threshold for LSIL and overlapping lesions (RCI scores up to 4) fell to 56.6% when the cut-off was raised to the high-grade lesion, CIN2+ (RCI 5+). Conversely, the specificity increased for HSIL. The positive likelihood ratio (+LR) increased from 2.7 to 4.64, and the -LR increased from 0.29 to 0.52. Although for cytology the trend was the same, baseline magnitudes were higher, while the range was significantly lower. The sensitivity of cytology at the HSIL cut-off fell to 89.6% vs. 56.6% for colposcopy, +LR increased from 2.21 to 3.23, and -LR increased from 0.08 to 0.16.

Calculation of interrater agreements between the grouped histology and colposcopy findings resulted in 55.3% for *n* 94 and 52.2% and 60.4% in specialists, respectively. Overall concordance between the colposcopic performance and histology reached 56.8% for benign and dubious lesions up to LSIL; 30.8% for overlapping lesions, up to CIN2; and 61.5% for HSIL. Accordingly, linear weighted Cohen's κ was found to be 0.47 ± 0.06 (95% CI 0.38;0.61); for specialist-1 0.34 ± 0.13 (95% CI 0.08;0.59), and 0.58 ± 0.1 (95% CI 0.39;0.81) for specialist-2. Meanwhile, in the mentioned study on cytology aspects of current cervical screening in the country (Balmagambetova et al., 2020), κ 0.62 ± 0.08 for azur-eosin staining was established.

3.2. Probability for HSIL development at viral load raising

To evaluate the probability of HSIL development at viral load increments within each RCI group, we designed a logistic regression model (Nagelkerke's R^2 0.44). The odds ratios (ORs) are presented in Table 3.

Table 1
Descriptive statistics of the study.

Colposcopic findings (grouped)	*n* (%), mean age (range), age of sexual debut (range)		*Cytology grades, %		Average viral load by groups (range)	*n* (%) and average viral load (GE*10^3 per sample) in each subgroup	HPV 16/18, %
	HPV - neg. *n* 846	HPV - pos. *n* 283	HPV - neg. *n* 846	HPV - pos. *n* 283			
Group 1 (Reid 0–2), *n* 710	*n* 611 (72.2%) 31.8 ± 8.7 (18–63) 20.8 ± 3.3 (17–27)	*n* 99 (34.9%) 30.5 ± 8.7 (19–61) 20.9 ± 2.8 (13–26)	NILM 62.6% ASCUS/AGC-NOS 30.1% LSIL 7.3% HSIL 0.0%	NILM 47.5% ASCUS/ AGC-NOS 34.7% LSIL 16.8% HSIL 1.0%	4.7 ± 2.5 (0.9–13.0)	Low – 2.4 ± 0.3 (*n* 28, 28.7%) Moderate – 3.8 ± 0.6 (*n* 36, 35.6%) High – 7.6 ± 1.9 (*n* 35, 34.7%)	23.7/6.9
Group 2 (Reid 3–4), *n* 343	*n* 200 (23.6%) 35.3 ± 9.8 (17–62) 20.6 ± 3.5 (15–25)	*n* 143 (50.5%) 34.8 ± 7.3 (17–58) 20.5 ± 3.3 (14–24)	NILM 48.8% ASCUS/AGC-NOS 32.2% LSIL 16.2% HSIL 2.1%	NILM – 49.3% ASCUS/ AGC-NOS 32.4% LSIL 16.7% HSIL 2.3%	5.9 ± 4.2 (1.0–22.3)	Low – 2.3 ± 0.5 (*n* 27, 18.9%) Moderate – 3.8 ± 0.5 (*n* 52, 36.4%) High – 9.2 ± 4.3 (*n* 64, 44.7%)	26.9/5.5
Group 3 (Reid 5+), *n* 76	*n* 35 (4.1%) 42.4 ± 9.4 (24–61) 21.7 ± 5.2 (14–27)	*n* 41 (14.5%) 41.9 ± 8.4 (22–63) 20.7 ± 2.7 (16–24)	NILM 42.9% ASCUS/AGC-NOS 35.7% LSIL 11.9% HSIL 9.5%	NILM – 23.2% ASCUS/ AGC-NOS 2.6%; LSIL 20.9% HSIL 23.3%	7.0 ± 4.6 (1.2–21.1)	Low – 2.0 ± 0.8 (*n* 5, 12.2%) Moderate – 4.1 ± 0.7 (*n* 8, 19.5%) High – 9.0 ± 4.4 (*n* 28, 68.3%)	37.2/9.3

* Cytology grades: NILM - Negative for intraepithelial lesions or malignancy; ASCUS - Atypical squamous cells of undetermined significance, cannot exclude (ASC-H); AGC-NOS - Atypical Glandular Cells Not Otherwise Specified; CIN - Cervical Intraepithelial Neoplasia; LSIL - Low-Grade Squamous Intraepithelial Lesions; HSIL - High-Grade Squamous Intraepithelial Lesions (Terminology Bethesda System, 2001).

Table 2
Coordinates of the obtained ROC curve with 95% CI.

Cut-off	Colposcopy (RCI scores)				Cytology			
	Sensitivity	Specificity	PPV*	PVN*	Sensitivity	Specificity	PPV	PVN
≥2 LSIL, overlapping lesion up to CIN2	81.6 (64.7;90.2)	72.6 (56.2;82.5)	70.0 (55.2;82.2)	80.0 (65.4;90.4)	95.5 (84.5;99.8)	57.9 (43.2;71.6)	66.5 (52.7;77.8)	94.5 (78.5;99.0)
≥3 HSIL (CIN2+)	56.6 (38.9;69.6)	88.3 (76.1;95.5)	80.0 (61.4;82.2)	69.2 (56.6;80.1)	89.6 (75.4;96.2)	74.5 (58.3;86.1)	74.6 (59.7;85.7)	89.1 (74.4;96.5)

*PPV - positive predictive value.
*PVN - negative predictive value.

As shown in Table 3, the chance for HSIL appearance rises depending on HPV load magnitudes within each group, thus promoting further deterioration of the cervix condition and related RCI. The ORs for high-grade lesion occurrence at viral load rising, for the group with scores by RCI up to 2 (supposedly, from NILM to CIN1) compared to women having severe colposcopic opinions, resulted in 3.4 vs. 39.7 ($p < 0.0001$).

4. Discussion

Commonly, the risk of developing CIN conjugately rises as the viral load of highly carcinogenic HPV types increases. Moreover, reportedly, there are type-specific differences in correlations with the severity of lesions. Dong et al. (2018) found that the viral loads of HPV-16, -31, -33, -52, and -58 positively correlated with the severity of cervical lesions, whereas those of HPV-18, -45, -56, -59, and other types did not. In our study, we did not separate the viral load by type.

To reveal the relationship between the colposcopy findings depending on viral loads, we determined the chance for HSIL development per RCI group. Probability for HSIL occurrence increased consecutively at viral load raising (ORs raised from 3.4 in group-1 up to 39.7, $p < 0.0001$ in the group with RCI 5+). Our findings turned out to be significantly inferior to those of other authors made based on a larger number of observations. Basu et al. (2018) established ORs for CIN2+ diagnosis in women with a high level of viral load as 46, 217.4, and 3915.1 for the "probable high grade" group, respectively ($p < 0.001$, *n* 39,728).

Overall, we established cytology superiority over colposcopy in CIN detection. We found acceptable sensitivity of colposcopy for detecting LSIL (81.6%) but observed a further decrease to 56.6% at raising the threshold to HSIL. Kushwah and Kushwah (2017), comparing the performance of Swede score and RCI, established the sensitivity of RCI as 89% for any lesion, similarly falling to 56% for HSIL, while the specificity increased to 92.9%. Alan et al. (2020), who also studied the Swede scoring system performance, found that a cut-off value ≥ 6 had a high sensitivity for high-grade lesions, and this scoring system was a useful tool for evaluating atypical cervical cytology in women with high-risk HPV infection.

We cannot explain the similarity of our data concerning the low sensitivity of RCI for the HSIL vs. the Swede system. Nevertheless, further research comparing the performance of the two scoring systems appears not to be justified, because the data obtained in this study suggests the need for globally adopted primary HPV testing implementation.

5. Conclusion

Our results are in line with a common trend stating that colposcopy alone is insufficient to provide proper detection and prediction of high-grade cervical lesions. In general, we established that the utility of colposcopy diagnosis through RCI pertains mostly to low-grade lesions. In addition, the probability of HSIL occurrence increases with increasing

Table 3
Effect of viral load within the colposcopy groups on HSIL development.

Colposcopy groups by RCI scores	HSIL	HPV viral load	*n* assessed	OR (95% CI)	p-value
Group 1 (RCI up to 2), HPV-negative cases	0.0%	–	611 (54.1%)	–	–
Group 1 (RCI up to 2), HPV-positive cases	7 (1.0%)	Low ($<10^3$ GE/ per sample)	28 (28.7%)	1.77 (0.59;5.25)	0.31
		Moderate ($10^3 - 10^5$ GE)	36 (35.6%)	1.9 (0.63;5.64)	0.25
		High ($>10^5$ GE)	35 (34.7%)	3.4 (1.7;6.7)	0.001
Group 2 (RCI up to 4)	8 (2.3%)	Low ($<10^3$ GE)	27 (18.9%)	1.9 (0.63;5.65)	0.25
		Moderate ($10^3 - 10^5$ GE)	52 (36.4%)	2.3 (0.96;5.34)	0.063
		High ($>10^5$ GE)	64 (44.7%)	5.3 (2.4;11.8)	<0.0001
Group 3 (RCI 5+)	10 (23.3%)	Low ($<10^3$ GE)	5 (12.2%)	5.3 (1.0;28.1)	0.045
		Moderate ($10^3 - 10^5$ GE)	8 (19.5%)	8.57 (3.8;19.5)	<0.0001
		High ($>10^5$ GE)	28 (68.3%)	39.7 (7.8;202.9)	<0.0001

HPV viral load.

Overall, colposcopy efficiency in the diagnosis of cervical precancerous lesions failed to show satisfactory reliability. Globally adopted primary HPV screening would be the best option despite the related costs.

CRediT authorship contribution statement

Saule Balmagambetova: Conceptualization, Project administration, Writing - original draft. **Andrea Tinelli:** Conceptualization, Writing - review & editing, Supervision. **Olzhas Urazayev:** Formal analysis, Methodology. **Arip Koyshybaev:** Formal analysis, Writing - review & editing. **Elnara Ismagulova:** Formal analysis, Data curation, Validation. **Kanshaiym Sakiyeva:** Data curation, Validation. **Saganaj Djussembekov:** Data curation, Validation. **Dinara Zholmukhamedova:** Data curation, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Consent for publication

Written informed consent was obtained from all the participants for publication this retrospective cohort study. Copies of written consent are available for review by the Editor-in-Chief of this journal on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2020.100661.

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