EVALUATION OF THE RISK OF CERVICAL INTRAEPITHELIAL NEOPLASIA PROGRESSION BASED ON CELL PROLIFERATION INDEX, EPITHELIAL-MESENCHYMAL TRANSITION AND CO-INFECTIONS

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Cervical cancer is the second most common gynaecological malignancy worldwide [15]"ISSN":"1542-4863 (Electronic. Human papilloma virus (HPV) infection, especially with high risk types, represent the major etiological factor for the development of cervical precancerous and cancerous lesions [7,17]. Cervical precancerous lesions are represented with the spectrum of cervical intraepithelial neoplasia (CIN), grade I-III (CINI-CINIII) [12]. About 60% of CINI lesions undergo regression after one year and does not progress into higher grade disease [13]. It has been suggested that various epithelial and microenvironment characteristics play a role in the progression in the progression of CIN. Recently, it has been also shown that infectious agents, other than HPV also play an important role in the development of higher grades of CIN and cervical cancer [10].

Several studies indicated that epithelial cell proliferation index is related to the progression of cervical intraepithelial neoplasia [2]. For example, the expression of proliferation marker Ki67 was significantly increased with the progression of CIN, from normal cervical epithelium, through CIN to carcinoma [18]. On the other hand, it has been shown that another proliferation marker Cyclin D1 is gradually decreased in the progression from CINI to CINIII. Whilst, it is increased in cervical carcinoma [16].

During the progression epithelial cancers, including cervical cancer, usually lose their epithelial characteristics and gain mesenchymal phenotype. This multi-step process is called epithelial-mesenchymal transition (EMT) [9]. On immunohistochemical level EMT is characterised with the loss of epithelial markers and higher expression of mesenchymal markers. Multiple studies have shown that EMT plays an important role in epithelial tumor progression and in the development of metastases [5]. However, the role of EMT in the progression of cervical intraepithelial neoplasia is not very well studied.

The aim of our current study was to analyse the markers of cervical intraepithelial neoplasia progression, including cell proliferation markers (Ki67, cyclin D1, phosphohistone H3) and epithelial-mesenchymal transition markers (E-kadherin, P63, β-catenin, vimentin). In addition, we have also analysed the role of co-infections in the progression of CIN disease including *bacterial vaginosis, chlamydia trachomatis and candida albicans.*

Material and methods. Formalin fixed and paraffin embedded tissue material was retrieved from the Research, Diagnostic and Teaching Laboratory of Tbilisi State Medical University, Georgia. Study included altogether 150 tissue samples, divided into two major groups: cases without co-infections (n=64) and cases with co-infections (n=86). Co-infections included *bacterial vaginosis, chlamydia trachomatis and candida albicans.* Cases without co-infections were divided into following subgroups: normal cervix (10 cases), CINI (18 cases), CINII (14 cases), CINIII (7 cases), invasive carcinoma (5 cases); Cases with co-infections were divided into following subgroups: cervix with only infections (15 cases), CINI (29 cases), CINII (19 cases), CINIII (15 cases), invasive carcinoma (8 cases);

4μ FFPE tissue sections were deparaffinized in xylene, rehydrated by using serial dilutions of ethanol (96%, 80%, 70%) and heat mediated antigen retrieval has been performed. Ready to use antibodies against the following antigens were used: Ki67 (K2), Cyclin D1 (polyclonal) and phosphohistone-H3 (pHH3), E-cadherin(36B5), p63 (7JUL), β-catenin (17C2), vimentin (V9) (Novocastra). Staining and visualisation has been performed using Bond polymer refine detection system. The number of positive cells were counted in 20HPF and cell proliferation index was calculated – based on Ki67, cyclin D1 and phosphohistone-H3 labelling. Proliferation index based on Ki67 and cyclin D1 labelling 0-15% was considered as low and >15% was considered as high. The positivity for E-cadherin, p63, β-catenin and vimentin was counted as percentage of positive atypical/tumor cells and the number of positive cells was varied between 0 and 100%.

Comparisons between groups were made using Kruskal-Wallis test. The Kruskal-Wallis test is a nonparametric (distribution free) test, and is used when the assumptions of one-way ANOVA are not met. The Kruskal-Wallis test can be used for both continuous and ordinal-level dependent variables. Correlations were assessed using Spearman's rank correlation. The Spearman's rank correlation is also used when data is nonparametrically distributed. P values <0.05 were considered as significant. All statistical tests were performed using SPSS software V19.00.

Results and discussion. The average Ki67 labelling index (%) in normal cervix without infections was 4±1.2, in CINI with co-infections it was 13±5.2, in CINII it was 15±6.9, in CINIII it was 20±5.1 and in invasive carcinoma it was 35±10.6. The average cyclin D1 labelling index in normal cervix without infections was 22±5.3, in CINI with co-infections it was 16±4.8, in CINII it was 12±3.6, in CINIII it was 9±2.6 and in invasive carcinoma it was 30±8.7. The average phosphohiston-H3 labelling index (%) in normal cervix without infections was 1±0.2, in CINI with co-infections it was 3±1.1, in CINII it was 6±1.9, in CINIII it was 9±2.1 and in invasive carcinoma it was 15±3.5.

Table 1. Distribution of Ki67, cyclin D1 and phosphohiston-H3 labelling index in groups without co-infections

	Without co-infection			Total N
	Ki67	Cyclin D1	Phosphohiston-H3	
Normal cervix	4±1.2	22±5.3	1±0.2	10
CINI	13±5.2	16±4.8	3±1.1	18
CINII	15±6.9	12±3.6	6±1.9	14
CINIII	20±5.1	9±2.6	9±2.1	7
Invasive CA	35±10.6	30±8.7	15±3.5	5

Table 2. Distribution of Ki67, cyclin D1 and phosphohiston-H3 labelling index in groups with co-infections

	With co-infection			Total N
	Ki67	Cyclin D1	Phosphohiston-H3	
Cervix with Infection	6±1.9	23±5.6	2±1.2	15
CINI	17±6.3	17±3.4	4±1.4	29
CINII	21±7.8	14±4.7	7±1.7	19
CINIII	26±8.2	10±3.7	11±2.9	15
Invasive CA	42±10.7	34±9.8	17±3.9	8

Table 3. Distribution of Ki67 and cyclin D1 labelling index in groups without co-infections

	Without co-infection				Total N
	Ki67		Cyclin D1		
	≤15	>15	≤15	>15	
Normal cervix	10	0	2	8	10
CINI	12	6	13	5	18
CINII	4	10	12	2	14
CINIII	2	5	4	3	7
Invasive CA	1	4	1	4	5

The average Ki67 labelling index (%) in cervix with infections was 6±1.9, in CINI it was 17±6.3, in CINII it was 21±7.8, in CINIII it was 26±8.2 and in invasive carcinoma it was 42±10.7. The average cyclin D1 labelling index in cervix with infections was 23±5.6, in CINI it was 17±3.4, in CINII it was 14±4.7, in CINIII it was 10±3.7 and in invasive carcinoma it was 34±9.8. The average phosphohiston-H3 labelling index (%) in cervix with infections was 2±1.2, in CINI it was 4±1.4, in CINII it was 7±1.7, in CINIII it was 11±2.9 and in invasive carcinoma it was 17±3.9.

High Ki67 labelling index (>15%) was detected in 0/10 (0%) cases of normal cervix without infections, whilst in 10/10 (100%) cases of normal cervix without infections Ki67 labelling index was low (≤15%). In CINI without co-infections Ki67 labelling index was low in 12/18 (66.7%) cases and it was high in 6/18 (33.3%) cases. In CINII without co-infections Ki67 labelling index was low in 4/14 (28.6%) cases and it was high in 10/14 (71.4%) cases. In CINIII without co-infections Ki67 labelling index was low in 2/7 (28.6%) cases and it was high in 5/7 (71.4%) cases. In invasive carcinoma without co-infections Ki67 labelling index was low in 1/5 (20%) cases and it was high in4/5 (80%) cases.

High cyclin D1 labelling index (>15%) was detected in 8/10 (80%) cases of normal cervix without infections, whilst in 10/10 (20%) cases of normal cervix without infections cyclin D1 labelling index was low (≤15%). In CINI without co-infections cyclin D1 labelling index was low in 13/18 (72.2%) cases and it was high in 5/18 (27.8%) cases. In CINII without co-infections cyclin D1 labelling index was low in 2/14 (14.3%) cases and it was high in 2/14 (85.7%) cases. In CINIII without co-infections cyclin D1 labelling index was low in 4/7 (57.1%) cases and it was high in 3/7 (42.9%) cases. In invasive carcinoma without co-infections cyclin D1 labelling index was low in 1/5 (20%) cases and it was high in 4/5 (80%) cases.

High Ki67 labelling index (>15%) was detected in 2/15 (13.3%) cases of cervix with infections, whilst in 13/15 (86.7%) cases of normal cervix with infections Ki67 labelling index was low (≤15%). In CINI with co-infections Ki67 labelling index was low in 17/29 (58.6%) cases and it was high in 12/29 (41.3%) cases. In CINII with co-infections Ki67 labelling index was low in 5/19 (26.3%) cases and it was high in 14/19 (73.7%) cases. In CINIII with co-infections Ki67 labelling index was low in 3/15 (20%) cases and it was high in 12/15 (80%) cases. In invasive carcinoma with co-infections Ki67 labelling index was low in 1/8 (12.5%) cases and it was high in 7/8 (87.5%) cases.

High cyclin D1 labelling index (>15%) was detected in 12/15 (80%) cases of cervix with infections, whilst in 3/15 (20%) cases of cervix with infections cyclin D1 labelling index was low (≤15%). In CINI with co-infections cyclin D1 labelling index was low in 22/29 (75.9%) cases and it was high in 7/29

Table 4. Distribution of Ki67 and cyclin D1 labelling index in groups with co-infections

	With co-infection				Total N
	Ki67		Cyclin D1		
	≤15	>15	≤15	>15	
Cervix with Infection	13	2	3	12	15
CINI	17	12	22	7	29
CINII	5	14	13	6	19
CINIII	3	12	11	4	15
Invasive CA	1	7	3	5	8

(24.1%) cases. In CINII with co-infections cyclin D1 labelling index was low in 13/19 (68.4%) cases and it was high in 6/19 cases (31.6%). In CINIII with co-infections cyclin D1 labelling index was low in 11/15 (73.3%) cases and it was high in 4/15 (26.7%) cases. In invasive carcinoma with co-infections cyclin D1 labelling index was low in 3/8 (37.5%) cases and it was high in 5/8 (62.5%) cases.

In normal cervix without infections average p63 expression (%) was 43±4.4, in CINI without co-infection average p63 expression was 52±6.2, in CINII without co-infection average p63 expression was 57±4.7, in CINIII without co-infection average p63 expression was 65±5.5 and in invasive carcinoma without co-infection average p63 expression was 38±3.9. In normal cervix without infections average E-cadherin expression (%) was 95±3.8, in CINI without co-infection average E-cadherin expression was 78±4.6, in CINII without co-infection average E-cadherin expression was 67±4.4, in CINIII without co-infection average E-cadherin expression was 56±4.8 and in invasive carcinoma without co-infection average E-cadherin expression was 33±2.6. In normal cervix without infections average β-catenin expression (%) was 43±3.6, in CINI without co-infection average β-catenin expression was 39±2.5, in CINII without co-infection average β-catenin expression was 30±4.6, in CINIII without co-infection average β-catenin expression was 24±4.1 and in invasive carcinoma without co-infection average β-catenin expression was 16±2.9. In normal cervix without infections, CINI without infections and CINII without infections vimentin was completely negative. In CINIII without co-infections the average vimentin expression was 17±4.8 and in invasive carcinoma without co-infections the average vimentin expression was 45±6.2.

In normal cervix with infections average p63 expression (%) was 47±5.6, in CINI with co-infection average p63 expression was 57±4.8, in CINII with co-infection average p63 expression was 60±5.5, in CINIII with co-infection average p63 expression was 67±6.3 and in invasive carcinoma with co-infection average p63 expression was 37±4.2. In normal cervix with infections average E-cadherin expression (%) was 88±2.3, in CINI with co-infection average E-cadherin expression was 74±4.5, in CINII with co-infection average E-cadherin expression was 63±2.9, in CINIII with co-infection average E-cadherin expression was 45±5.6 and in invasive carcinoma with co-infection average E-cadherin expression was 27±2.4. In normal cervix with infections average β-catenin expression (%) was 41±2.2, in CINI with co-infection average β-catenin expression was 35±5.1, in CINII with co-infection average β-catenin expression was 29±4.5, in CINIII with co-infection average β-catenin expression was 26±2.4 and in invasive carcinoma with co-infection average β-catenin expression was 21±2.6. In normal cervix with infections, CINI without infections and CINII with infections vimentin was completely negative. In CINIII with co-infections the average vimentin expression was 19±5.7 and in invasive carcinoma with co-infections the average vimentin expression was 36±4.8.

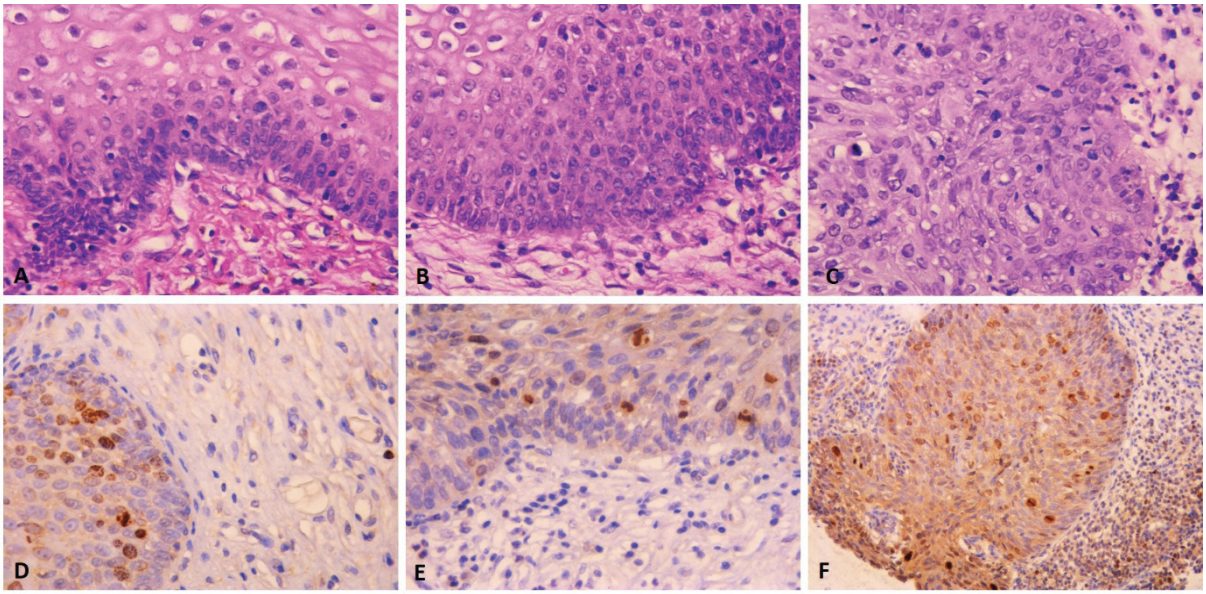


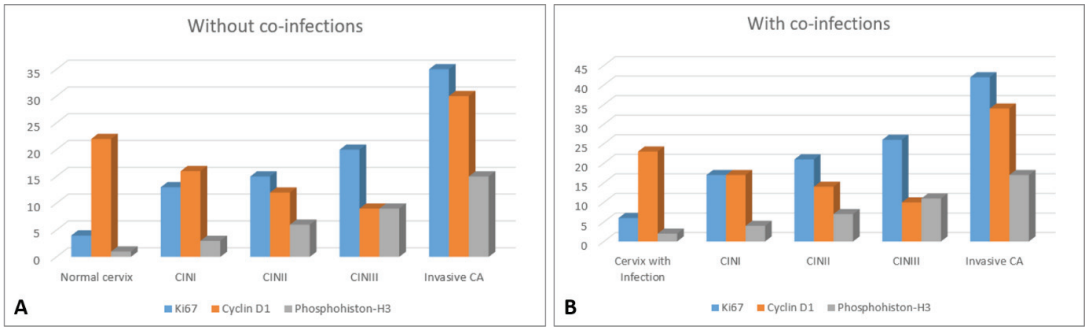
Fig. 1. A. CINI, H&E, x200, B. CINII, H&E, x200, C. CINIII, H&E, x200, D. Ki67 expression in CINII, IHC, x200, E. phosphohiston-H3 expression in CINII, IHC, x200, F. p63 expression in CINIII, IHC, x200

Table 5. Distribution of p63, E-cadherin, β-catenin and vimentin in groups without co-infections

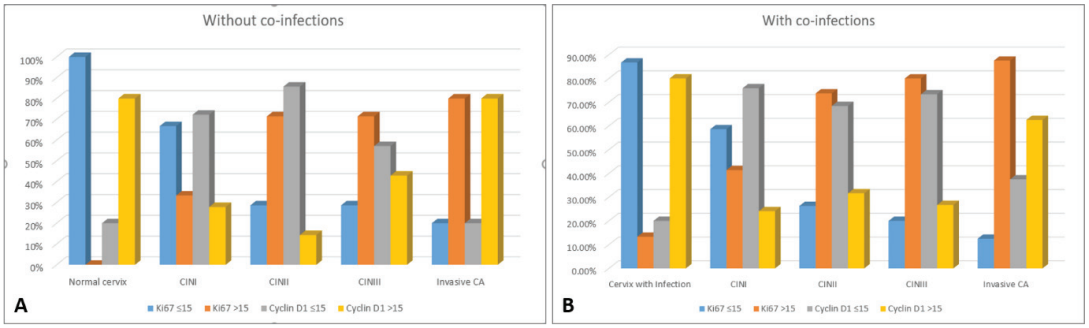
	Without co-infection				Total N
	p63	E-cadherin	β-catenin	vimentin	
Normal cervix	43±4.4	95±3.8	43±3.6	0	10
CINI	52±6.2	78±4.6	39±2.5	0	18
CINII	57±4.7	67±4.4	30±4.6	0	14
CINIII	65±5.5	56±4.8	24±4.1	17±4.8	7
Invasive CA	38±3.9	33±2.6	16±2.9	45±6.2	5

Table 6. Distribution of p63, E-cadherin, β-catenin and vimentin in groups with co-infections

	With co-infection				Total N
	p63	E-Cadherin	β-catenin	vimentin	
Cervix with Infection	47±5.6	88±2.3	41±2.2	0	15
CINI	57±4.8	74±4.5	35±5.1	0	29
CINII	60±5.5	63±2.9	29±4.5	0	19
CINIII	67±6.3	45±5.6	26±2.4	19±5.7	15
Invasive CA	37±4.2	27±2.4	21±2.6	36±4.8	8



Graph 1. A. Distribution of Ki67, cyclin D1 and phosphohiston-H3 labelling index in groups without co-infections; B. Distribution of Ki67, cyclin D1 and phosphohiston-H3 labelling index in groups with co-infections

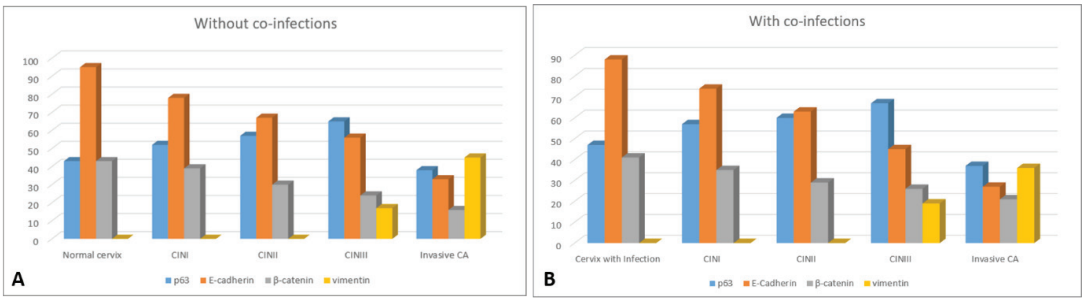


Graph 2. A. Distribution of Ki67 and cyclin D1 labelling index in groups without co-infections; B. Distribution of Ki67 and cyclin D1 labelling index in groups with co-infections

The distribution of Ki67 labelling index in groups showed that the lowest expression of Ki67 is present in normal cervix without infections. In cervix with infections Ki67 labelling index is slightly higher. The expression of Ki67 labelling index is gradually and significantly increases with the progression of cervical intraepithelial neoplasia and reaches it's maximum expression level in invasive cervical carcinoma. The comparison of Ki67 labelling index in groups with and without co-infections showed that Ki67 expression is significantly higher in all groups with co-infections compared to groups without co-infections. Cyclin D1 labelling index is highest in normal cervix and in invasive carcinoma, it is gradually decreased in CINI to CINIII lesions. The comparison of cyclin D1 labelling index in groups with and without co-infections did not show a significant difference. Phosphohiston-H3 expression was also significantly increased from normal cervix to CINI to CINIII and invasive carcinoma in both groups with infections and without infections. However, the expression of phoshohiston-H3 was not different in groups with and without infections.

We have further analysed the cases with low and high proliferation index based on Ki67 and cyclin D1 labelling. The number of cases with high Ki67 labelling index (>15%) was significantly increased with the progression if CIN disease. The comparison of Ki67 labelling index in groups with and without co-infections showed that, the number of cases with high Ki67 labelling index was significantly higher in groups with co-infections compared to groups without co-infections. With the difference from Ki67 the cyclin D1 expression showed the opposite trend. It was significantly decreased with the progression of CIN disease. However, it was significantly higher in invasive carcinoma.

Kim and colleagues showed that Ki67 positivity is associated with the progression of cervical intraepithelial neoplasia, whilst the negativity of Ki67 was associated with the regression of CIN disease [8]. Our findings are in line with the findings of Kim at al., similar to their study, we have also found that Ki67 labelling index is significantly higher in higher grades of CIN and in invasive carcinoma. Several studies also investigated the role of cyclin D1 expression in the progression of cervical intraepithelial neoplasia. Wang et al., showed that cyclin D1 expression is significantly decreased during the progression of CIN [9], which is in line to our study results. Similar to our study results, Bae and colleagues also found significantly decreased expression of cyclin D1 from CINI to CINIII disease, whilst it was significantly higher in invasive carcinoma of the cervix [1]. Brustmann and colleagues found a significant increase of phosphohistone-H3 expression during the CIN progression [4]. Their study results are in line with our study results, as we also found a sharp increase of phosphohiston-H3 expression from normal cervix to CINI to CINIII and invasive carcinoma.



Graph 3. A. Distribution of p63, E-cadherin, β-catenin and vimentin in groups without co-infections; B. Distribution of p63, E-cadherin, β-catenin and vimentin in groups with co-infections

Expression of epithelial marker p63 was also significantly increased during the progression of CIN disease, whilst the expression of E-cadherin and β-catenin was significantly decreased. The comparison of these markers in groups with and without infections showed that p63 and β-catenin does not differ. However, the expression of E-cadherin was significantly decreased in groups with co-infections compared to groups without co-infections. Rajic and colleagues showed that the *chlamydia trachomatis* infection is associated with the decrease of E-cadherin expression via promoter hypermethilation [14], which is in line to our study results. In addition, similar to our study, several studies also indicated the decreased expression of E-cadherin and β-catenin during the progression of cervical intraepithelial neoplasia [3,6] and increased expression of p63 [11]. Vimentin is a mesenchymal marker which is showed to be increased during epithelial mesenchymal transition. In our study groups, vimentin was not expressed in normal cervix in CINI and in CINII neither in cases with infections nor without infections, whilst vimentin was expressed in CINIII and in higher degree in invasive carcinoma cases, which indicates that epithelial mesenchymal transition plays an important role in the progression of cervical intraepithelial neoplasia to invasive carcinoma. Similar to our study results, Jiang et al., also found an increased expression of vimentin during the progression of cervical intraepithelial neoplasia [6].

Conclusions. Our study results indicate that the measurement of proliferation index, based on Ki67 labelling, as well as mitotic index based on phosphohiston-H3 detection can reliably indicate high and low risk groups of the progression of cervical intraepithelial neoplasia. Similarly, higher p63 expression, loss of E-cadherin and β-catenin and higher vimentin expression can indicate the progression risk of cervical intraepithelial neoplasia. The presence of co-infections is associated with the increased expression of proliferation marker Ki67 and the loss of E-cadherin and therefore it can be considered as an additional marker of CIN progression.

REFERENCES

1. Bae D.S. et al. Aberrant expression of cyclin D1 is associated with poor prognosis in early stage cervical cancer of the uterus. // Gynecol. Oncol. 2001; vol. 81, no. 3: pp. 341–347.
2. Balasubramaniam S.D., Balakrishnan V., Oon C.E., Kaur G. Key Molecular Events in Cervical Cancer Development. // Medicina (Kaunas).2019; 55(7): 384.
3. Branca M. et al. Down-regulation of E-cadherin is closely associated with progression of cervical intraepithelial neoplasia (CIN), but not with high-risk human papillomavirus (HPV) or disease outcome in cervical cancer. // Eur. J. Gynaecol. Oncol.2006; vol. 27, no. 3:215–223.
4. Brustmann M.I.H. Immunodetection of phosphohistone H3 (PHH3) in cervical squamous neoplasia. // Pathology 2014; vol. 46, no. 2: S109.
5. Diepenbruck M., Christofori G. Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? // Curr. Opin. Cell Biol. 2016; vol. 43, pp. 7–13.
6. Jiang J., Li X., Yin X., Zhang J., Shi B. Association of low expression of E-cadherin and beta-catenin with the progression of early stage human squamous cervical cancer.," Oncol. Lett.2019; vol. 17, no. 6: 5729–5739.
7. Kann H., Hortlund M., Eklund C., Dillner J., Faust H. Human Papillomavirus types in cervical dysplasia among young HPV-vaccinated women: Population-based nested case-control study. // Int. J. Cancer, Dec. 2019.
8. Kim S.M., Lee J.U., Lee D.W., Kim M.J., Lee H.N. The prognostic significance of p16, Ki-67, p63, and CK17 expression determined by immunohistochemical staining in cervical intraepithelial neoplasia 1. // Korean J. Obstet.2011; vol. 54, no. 4.
9. Lee MY, Shen MR. Epithelial-mesenchymal transition in cervical carcinoma. // Am J Transl Res – 2012. – V. 4. – №1 : 1-13.
10. Liang, Y., Chen, M., Qin, L. et al. A meta-analysis of the relationship between vaginal microecology, human papillomavirus infection and cervical intraepithelial neoplasia. // Infect Agents Cancer 14, 29 (2019). https://doi.org/10.1186/s13027-019-0243-8
11. Mitildzans A., Arechvo A., Rezeberga D., Isajevs S. Expression of p63, p53 and Ki-67 in Patients with Cervical Intraepithelial Neoplasia. // Turk Patoloji Derg. 2017; vol. 33, no. 1: 9–16.
12. Montz F.J. Management of high-grade cervical intraepithelial neoplasia and low-grade squamous intraepithelial lesion and potential complications. //Clin. Obstet. Gynecol. 2000; 43(2):.
13. Ostor A.G. Natural history of cervical intraepithelial neoplasia: a critical review. // Int. J. Gynecol. Pathol. 1993; 12(2):pp. 186–192.
14. Rajic J. et al. Chlamydia trachomatis Infection Is Associated with E-Cadherin Promoter Methylation, Downregulation of E-Cadherin Expression, and Increased Expression of Fibronectin and alpha-SMA-Implications for Epithelial-Mesenchymal Transition.//Front. Cell. Infect. Microbiol. 2017; vol. 7: p. 253.
15. Siegel R.L., Miller K.D., Jemal A. Cancer statistics 2019. // CA. Cancer J. Clin. 2019; 69(1): 7–34.
16. Wang P. et al. Expression of cyclin D1 in cervical intraepithelial neoplasia and squamous cell carcinoma and its relationship with HPV16 E7 gene. Zhonghua bing li xue za zhi. // Chinese J. Pathol. 2015; vol. 44, no. 12: 884–888.
17. Wojtyla C., La Vecchia C. Cervical cancer mortality in young adult European women. // Eur. J. Cancer 2020; 126:56–64.
18. Zhang X, Shen D. p16INK4a and Ki-67 measurement predict progression of cervical low-grade squamous intraepithelial lesion. // Int J Clin Exp Pathol. 2018;11(8):4109-4116.

SUMMARY

EVALUATION OF THE RISK OF CERVICAL INTRAEPITHELIAL NEOPLASIA PROGRESSION BASED ON CELL PROLIFERATION INDEX, EPITHELIAL-MESENCHYMAL TRANSITION AND CO-INFECTIONS

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Human papilloma virus (HPV) infection, especially with high risk types, represent the major etiological factor for the development of cervical precancerous and cancerous lesions. However, other factors including cell proliferation index, epithelial-mesenchymal transition and the presence of co-infections might also influence the progression of cervical intraepithelial neoplasia (CIN).

The aim of our study was to analyse, the expression of cell proliferation markers and epithelial-mesenchymal transition markers during the progression of cervical intraepithelial neoplasia, in cases with and without co-infections. Standard immunohistochemistry was used to detect, Ki67, cyclin D1, phosphohiston-H3, p63, E-cadherin, β-catenin and vimentin. The results of our study indicated that the expression of Ki67, phosphohiston-H3 and p63 is significantly increased during the progression of CIN disease, whilst the expression of E-cadherin and β-catenin are progressively lost.

The expression of mesenchymal marker vimentin is also increased in CINIII and in invasive carcinoma. Proliferation index based on Ki67 labelling is significantly higher in cases with co-infections and the expression on E-cadherin is significantly lower in cases with co-infections compared to cases without co-infections. In conclusion, the measurement of proliferation index, based on Ki67 labelling, as well as mitotic index based on phosphohiston-H3 detection can reliably indicate high and low risk groups of the progression of CIN. Similarly, higher p63 expression, loss of E-cadherin and β-catenin and higher vimentin expression can indicate the progression risk of CIN. The presence of co-infections is associated with the increased expression of proliferation marker Ki67 and the loss of E-cadherin and therefore it can be considered as an additional marker of CIN progression.

Keywords: cervical intraepithelial neoplasia, co-infections, marker of cervical intraepithelial neoplasia progression, CIN, Ki67, cyclin D1, phosphohiston-H3, p63, E-cadherin, β-catenin, vimentin.

РЕЗЮМЕ

ОЦЕНКА РИСКА ПРОГРЕССИРОВАНИЯ ИНТРАЭПИТЕЛИАЛЬНЫХ НЕОПЛАЗИЙ ШЕЙКИ МАТКИ НА ОСНОВЕ ПРОЛИФЕРАТИВНОГО ИНДЕКСА, ЭПИТЕЛИАЛЬНО-МЕЗЕНХИМАЛЬНОЙ ТРАНЗИЦИИ И КОИНФЕКЦИИ

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Инфицирование человеческим папилломавирусом, особенно подтипами высокого риска, является главным риск-фактором развития преканцерных и опухолевых повреждений шейки матки. Другие факторы, такие как пролиферативный индекс клеток, эпителиально-мезенхимальная транзиция и коинфекция также играют значимую роль в процессе прогрессии интраэпителиальных неоплазий шейки матки.

Целью исследования явилось определение маркеров пролиферации и эпителиально-мезенхимальной транзиции в процессе прогрессии интраэпителиальных неоплазий шейки матки при коинфекции и без ее наличия.

Стандартным иммуногистохимическим методом изучены молекулярные маркеры: Ki67, TLR9, циклин D1, фосфогистон-H3, p63, E-кадгерин, β-катенин и виментин. Результаты исследования показали, что эксперссия Ki67, фосфогистона-H3 и p63 значительно увеличивается в процессе прогрессии интраэпителиальных неоплазий шейки матки, тогда как эксперссия E-кадгерина и β-катенина прогрессивно теряется. Эксперссия мезенхимального маркера виментина также увеличивается в CINIII и инвазивных карциномах. В случаях с коинфекцией пролиферативный индекс по Ki67 значительно выше, а экспрессия E-кадгерина значительно ниже. Оценка индекса Ki67 и фосфогистона-H3 позволяет достоверно выделить высокие и низкие группы риска в процессе прогрессии интраэпителиальных неоплазий шейки матки, также как высокая экспрессия p63 и виментина, низкая экспрессия E-кадгерина и β-катенина. Наличие коинфекции возможно использовать как дополнительный риск-фактор в процессе прогрессии интраэпителиальных неоплазий шейки матки.

რეზიუმე

საშვილოსნოს ყელის ინტრაეპითელური ნეოპლაზიების პროგრესიის რისკის შეფასება პროლიფერაციული ინდექსის, ეპითელურ-მეზენქიმური ტრანზიციის და თანაინფექციების საფუძველზე

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თ.ასათიანი, გ.ბურკაძე

თბილისის სახელმწიფო სამედიცინო უნივერსტეტი, საქართველო

ადამიანის პაპილომავირუსით ინფიცირება, განსაკუთრებით მაღალი რისკის ქვეტიპებით, წარმოადგენს საშვილოსნოს ყელის პრეკანცერული და სიმსივნური დაზიანებების განვითარების მთავარ რისკ-ფაქტორს. თუმცა სხვა ფაქტორები, როგორებიცაა უჯრედების პროლიფერაციული ინდექსი, ეპითელურ-მეზენქიმური ტრანზიცია და თანაინფექციების არსებობა ასევე შესაძლებელია მნიშვნელოვან როლს თამაშობდნენ საშვილოსნოს ყელის ინტრაეპითელური ნეოპლაზიების პროგრესიის პროცესში. კვლევის მიზანს შეადგენდა პროლიფერაციული მარკერების და ეპითელურ-მეზენქიმური ტრანზიციის მარკერების შესწავლა საშვილოსნოს ყელის ინტრაეპითელური ნეოპლაზიების პროგრესიის პროცესში, ინფექციების თანაარსებობით და მათ გარეშე. სტანდარტული იმუნოჰისტოქიმიური გამოკვლევით შესწავლილია შემდეგი მარკერები: Ki67, ციკლინ D1, ფოსფოჰისტონ-H3, p63, E-კადჰერინი, β-კატენინი და ვიმენტინი. კვლევის შედეგებმა აჩვენა, რომ Ki67,ფოსფოჰისტონ-H3 და p63-ის ექსპრესია მნიშვნელოვნად იზრდება საშვილოსნოს ყელის ინტრაეპითელური ნეოპლაზიების პროგრესიის პროცესში, მაშინ როდესაც E-კადჰერინის და β-კატენინის ექსპრე-

სია პროგრესულად იკარგება. მეზენქიმური მარკერის ვიმენტინის ექსპრესია ასევე იზრდება CINIII-სა და ინვაზიურ კარცინომაში. თანაინფექციის მქონე შემთხვევებში პროლიფერაციული ინდექსი Ki67-ის მონიშვნის მიხედვით, გაცილებით უფრო მაღალია, ხოლო E-კადჰერინის ექსპრესია გაცილებით უფრო დაბალია. Ki67-ის ინდექსის და ფოსფოჰისტონ-H3-ის შეფასებით სარწმუნოდ არის შესაძლებელი საშვილოსნოს ყელის ინტრაეპითელური ნეოპლაზიის პროგრესიის მაღალი და დაბალი რისკ ჯგუფების გამოვლენა, ისევე როგორც, მაღალი p63-ის ექსპრესიით, დაბალი E-კადჰერინის და β–კატენინის და მაღალი ვიმეტინის ექსპრესიით. თანაინფექციების არსებობა შესაძლოა გამოყენებული იქნას როგორც საშვილოსნოს ყელის ინტრაეპითელური ნეოპლაზიების პროგრესიის რისკის განმსაზღვრელი დამატებითი რისკ-ფაქტორი.

KNEE JOINT STRUCTURAL CHANGES IN OSTEOARTHRITIS AND INJECTIONS OF PLATELET RICH PLASMA AND BONE MARROW ASPIRATE CONCENTRATE

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Osteoarthritis (OA) is the most common form of degenerative joint disease and one of the main causes of pain and disability in middle and elderly age patients [3]. Despite some advances in the study of pathogenesis of the disease and knee arthroplasty, there is still no effective specific treatment of osteoarthritis [4]. It is supposed that local administration of autologous cellular materials may contribute to the restoration of injured structures or reduce progressive damage to the knee joint. The use of platelet rich plasma (PRP) and bone marrow aspirate concentrate (BMAC) has rapidly widened over the last decade. Preparation of their concentrates above physiological indices is considered as a condition for stimulating recovery processes in the damaged area [8]. However, there are conflicting results on the effectiveness of these approaches, and some authors report on the absence of a recovery process under experimental conditions [2], and others conclude about the reduction of pain in mild and moderate osteoarthritis [1]. Most authors agree that autologous cell derivatives are not only safe to use, but can also potentially improve recovery both when using alone and in combination therapy [7]. Thus, Krych et al. in the analysis of the use of artificial cartilage showed better cartilage preservation after one year in case of additional administration of PRP or BMAC [5].

Animal models of osteoarthritis are often used to study the mechanisms of progressive degeneration of articular cartilage and to assess the effect of various drugs and cellular technologies on its prevention. The spontaneous development of osteoarthritis in experimental animals is long-lasting and is usually associated with age-related changes and other factors. The assessment of the potential effect of drugs requires the same nature of degenerative changes in all study cases. Thus, the model with cartilage defect, anterior cruciate ligament intersection and medial meniscus resection can cause rapid, topographically and morphometrically typical progressive damage to articular cartilage. In our study, we hypothesized that administration of PRP and BMAC may influence on the development of degenerative changes of articular cartilage of the knee joint in osteoarthritis.

The aim is to study the effect of PRP and BMAC on knee joint structural changes in rabbit osteoarthritis models.

Material and methods. The experiments were carried out on male Chinchilla rabbits weighing 2.47 [2.25-2.7] kg. The animals were kept in the vivarium of Shupyk National Medical Academy of Postgraduate Education with free access to water and food. All manipulations with the animals were conducted in compliance with (European Convention for the protection of vertebrate animals used for experimental and other scientific purposes, # 123, Council of Europe, L222, 24/08/1999, p. 31). Ethical approval for the research was obtained from the Ethics Committee of Shupyk National Medical Academy of Postgraduate Education, Approval No. 11 under the date of 11/19/2018.

Initially, the animals were randomly allocated in four groups: 1) control (intact); 2) osteoarthritis model + dual intra-articular injection of saline solution after 4 and 6 weeks; 3) osteoarthritis model + dual intra-articular injection of PRP after 4 and 6 weeks; 4) osteoarthritis model + intra-articular injection of BMAC after 4 weeks and PRP after 6 weeks. The experimental conditions in both knee joints in each animal were identical.

The animals were anesthetized with 35 mg/kg IM ketamine + 5 mg/kg IM xylazine to undergo the surgery. The knee joints were trimmed and treated with antiseptics. Medial parapatellar approach with patellar dislocation was performed. The osteoarthritis model consisted in the mechanical simulation of a standard cartilage defect of the medial femoral condyle, intersection of the anterior cruciate ligament, and resection of the medial meniscus and fat body (Fig. 1).

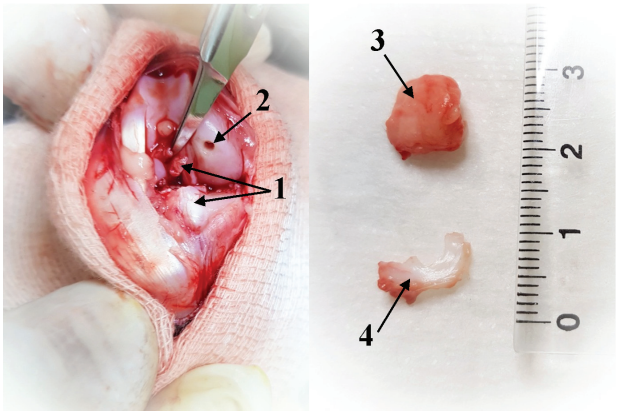

Fig. 1. Intraoperative view of the knee joint. 1 – anterior cruciate ligament stump; 2 – cartilage defect of the medial femoral condyle; 3 – removed fat body; 4 – removed medial meniscus

The wounds were sutured tightly with 3-0 threads (Vicryl, Ethicon Inc, USA) and subsequently treated with povidone iodine (Betadine, Egis, Hungary) daily till complete healing. The