Original Paper

Clinical and Morphological Study of Cervical Squamous Intraepithelial Lesions

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ABSTRACT: Squamous intraepithelial lesions (SILs) are cancer precursors targeted by secondary prevention of cervical cancer programs that are sometimes difficult to grade accurately. Mena is an actin regulatory protein involved in membrane protrusion, cell motility, in tumor invasion and metastasis. We studied retrospectively 68 cases of patients diagnosed with squamous intraepithelial lesions that received expedited treatment (treatment without colposcopic biopsy). We analyzed demographic, behavioral data, obstetrical and medical history, from the patients' medical charts and we studied the cervical fragments or cones harvested after the excisional procedure. Our study failed to identify a correlation between SILs and risk factors such as low socioeconomic status, combined oral contraceptive use, intrauterine device use, parity, gravity, except for the tobacco smoking habit that proved to be related to the cervical lesions' development. Mena was expressed in most of the analyzed SILs and its expression was correlated with lesions' grade in terms of both area and intensity, suggesting that Mena stains especially abnormal cells and that its expression intensity correlates with the risk of malignant transformation. Further studies are needed to validate Mena as an early stage of cervical carcinogenesis marker.

KEYWORDS: *Squamous intraepithelial lesions, Mena, risk of malignant transformation.*

Introduction

Cervical cancer, the fourth leading cause of cancer death among women worldwide [1], is the long term result of persistent infection by oncogenic human Papilloma virus (HPV) [2].

Although it is a necessary condition, HPV infection alone is not sufficient to cause a cervical malignancy [3].

Other factors like low socioeconomic status, tobacco smoking, long-term combined oral contraceptive use, IUD (intrauterine device) use, multiparity, multigravidity and HIV infection, have been associated with an increased risk of cervical precancerous lesions development and progression.

Squamous intraepithelial lesions (SILs), also referred to as cervical intraepithelial neoplasia (CIN), are cancer precursors and they represent proliferations of squamous cells, exhibiting viral cytopathic changes or maturation abnormalities, or both of them, features that do not project beyond the basement membrane.

They affect mostly women in their reproductive age [4].

In 2020, the World Health Organization [5] published the latest classification of female reproductive organ tumors, that uses a 2-tier grading system and divides cervical lesions into low-grade SILs (LSIL) (CIN 1) and high-grade SILs (HSIL), which encompasses CIN 2 (moderate CIN) and CIN 3 (severe CIN).

LSIL is considered the mark of the HPV infection, it is characterized by the abnormal cellular proliferation confined to the lower third of the epithelium and it regresses spontaneously in almost all cases.

The management of LSIL is observational [6] because these lesions may persist or, in rare instances, they may progress to HSIL (CIN 2).

HSIL presents nuclear abnormalities that extend above the lower third of epithelial thickness and they usually progress into an invasive carcinoma.

As a result, these lesions require excisional treatment.

Morphologically, CIN 2 maintains cytoplasmic maturation in the upper third of the epithelium, whereas CIN 3 presents no maturation difference across the epithelial layers.

While an accurate histopathological diagnosis may be challenging [7], latest guidelines regarding SILs' management accept different approaches of patients with HSIL (CIN 2) and of those diagnosed with HSIL (CIN 3) [6].

The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions (LAST) [8] recommends as ancillary testing evaluating p16 immunoexpression in cases where a diagnosis of HSIL (CIN 2) is entertained or HSIL is difficult to distinguish from mimickers and in cases of professional disagreement over the interpretation of histologic specimens.

The p16 protein is a cyclin-dependent kinase inhibitor, used as a surrogate marker for hrHPV infection and it is overexpressed in roughly all HSILs and some LSILs.

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However, LAST [8] advises against the routine use of p16 immunohistochemical staining in SILs diagnosis, given the significant proportion of unequivocal LSILs that present a p16 positive reaction [9] and the insufficiency of scientific evidence that p16 positive CIN 1 are more likely to progress, compared with p16 negative CIN 1.

Therefore, new predictive markers, able to distinguish the lesions which carry a high risk of progression from those with a low risk, may be used to increase the diagnosis accuracy, and further reduce the over-and undertreatment of SILs.

Mammalian-enabled (Mena) protein is a member of the proline-rich Enabled (Ena)/Vasodilator-Stimulated Phosphoprotein (VASP) family of actin nucleators and elongators, among VASP and Ena/VASP-like (Evl) [10].

By modulating the actin cytoskeletal polymerization in various cells, it plays a key role in membrane protrusion and cell motility and it is involved in tumor invasion and metastasis [11].

Recent studies showed that Mena is overexpressed in breast cancer [12], it regulates carcinoma cell invasion and promotes lung metastasis [13].

In addition, some authors report an increased Mena expression in gastric carcinoma [14], hepatocellular carcinoma [15], thyroid carcinoma [16], clear-cell renal cell carcinoma [10] and oral squamous cell carcinoma [17,18].

Moreover, Mena's expression was also reported in benign breast lesions that may be involved in breast carcinogenesis. However, the immunoexpression of Mena in cervical lesion is not enough studied [19].

We aimed to assess the Mena immunophenotype of premalignant squamous lesions of the cervix uteri and correlate it with clinical factors.

Materials And Method

We conducted a retrospective study which included 68 cervical samples from as many patients admitted on the Obstetrics and Gynecology Clinics of the Emergency County Hospital of Craiova, between 2018 and 2020 and diagnosed in the Pathology Department of the same hospital.

They were all subject to an excisional procedure (LLETZ-large loop excision of the transformation zone) performed for an abnormal cytologic finding without a confirmatory colposcopic biopsy (expedited treatment).

We analyzed demographic, behavioral data, obstetrical and medical history from the patients' medical charts: age at the time of the diagnosis, area of residence, tobacco smoking, COC use for more than 2 years and IUD use, number of pregnancies-gravidity, number of births-parity, HIV status and HPV vaccination status.

In addition, we studied material represented by cervical fragments or cones, that were fixed in 10% buffered formalin, processed by the classical histopathological technique and stained with hematoxylin and eosin.

The lesions were classified according to the latest World Health Organization (WHO) [5] recommendations.

For the morphologically ambiguous cases, p16 immunostaining was performed, in order to increase the diagnosis accuracy.

In this study, the immunoexpression of Mena was assessed at the epithelial level, where SILs abnormalities occur and in the adjacent stroma. Further, Mena's expression was followed in relation to the risk factors of cervical lesions identified in the selected cases.

Immunohistochemical assessment was performed on 4-μm sections mounted onto Superfrost slides.

The sections were deparaffinized using xylene and rehydrated in ethanol.

The antigen retrieval was done by microwaving the slides in citrate buffer (pH6) at 650W for 21 minutes.

After that, endogenous peroxidase was blocked with 1% hydrogen peroxide solution and nonspecific sites were blocked using 2% skimmed milk.

Next, Mena antibody, clone 21, provided by BD Biosciences, diluted 1:100 was added to the slides and they were refrigerated at 4°C for 18 hours.

The following day, the slides were washed thoroughly in PBS, and secondary biotinylated antibody was applied onto them. 30' later, Streptavidin-Horseradish peroxidase (HRP) was applied on the slides and they were incubated for another 30' at room temperature.

The development was made with substrate-chromogen solution 3,3'-diaminobenzidine dihydrochloride (DAB) for 2 minutes.

For nuclear counterstaining, we used Mayer's Hematoxylin.

Then, the samples were dehydrated, cleared, and mounted. Negative-control stainings were obtained by omitting the primary antibodies.

High-resolution Figures (10 on each case) were acquired with a Nikon 90i motorized

microscope (Nikon Europe BV, Amsterdam, the Netherlands) equipped with a plan apochromat high numerical aperture immersion objective (20x, NA=0.95), using the same exposure.

After we imported the Figures in Figure Pro Plus 6.0., a fixed threshold of the Mena signal was used to assess stained area and integrated optical density (IOD) at the epithelial level, which was manually delined from the rest of the sample.

Measurements data were exported in Microsoft Excel 2016 and the sum of the stained area and IOD were made for each capture.

Further, an average of the stained area and one for the IOD was performed for each case.

For the expression of Mena at the stromal level, positive cells were manually counted on 20×field areas-10 fields for each case, and an average number of cells was calculated for each case.

The averages of measurements for each case was compared using a one-way ANOVA test for multiple comparation within IBM SPSS (Statistical Package for the Social Sciences) Statistics 29.0.1.0 software and values of $p<0.05$ were considered significant.

For comparing the means of two groups we used the Two-Sample t-Test and for categorical data we used a chi-square (X^2) test within IBM SPSS (Statistical Package for the Social Sciences) Statistics 29.0.1.0 software and values of $p<0.05$ were considered significant.

The study was approved by the Local Ethics Committee, the written informed consent being obtained from the patients.

Results

This study included 68 cases diagnosed with squamous intraepithelial lesions.

In our study group, most cases were represented by HSIL (77,94%) which was the diagnosis of 53 patients aged between 26 and 59, with an average of 39,18.

In the LSIL group the age range was 26 to 54, with an average of 36,46 (±9,84), while in the HSIL group the age range was 27 to 59, with an average of 39,94 (±7,56).

There is no significant statistical correlation between mean age and diagnosis (t-Test, $p=0.0736$).

Regarding risk factors associated with the cervical lesions' development, we did not find a significant statistical correlation between any of the studied risk factors, except for the smoking habit, which was weekly correlated with the SILs diagnosis($p=0.041$).

We also analyzed distribution of the residential area of the patients as a surrogate for their socioeconomic status and we observed that most of them (78,19%) were living in an urban area.

None of the patients were HIV positive.

None of the subjects were vaccinated against HPV.

We summarized the patients' data in Table1.

Table 1. Main features of the patients included in this study.

			LSIL	HSIL	Total	*p* value (χ2 test)
Area of residence	Rural		1(1,47%)	6(8,82%)	7	0.60
	Urban		14(20,58%)	47(69,11%)	61	
Smoking			5 (7.35%)	33(48.52%)	38	0.041
COC use			6 (8.82%)	24 (35.29%)	30	0.71
IUD use			0 (0.00%)	5 (7.35%)	5	0.063
Parity		0	3 (4.41%)	6 (8.82%)	9	0.84
		1	7 (10.29%)	27 (39.71%)	34	
		2	4 (5.88%)	17 (25.00%)	21	
		3+	1 (1.47%)	3 (4.41%)	4	
Gravidity		0	2 (2.94%)	4 (5.88%)	6	0.45
		1	5 (7.35%)	13 (19.12%)	18	
		2	5 (7.35%)	16 (23.53%)	21	
		3	3 (4.41%)	10 (14.71%)	13	
		4+	0 (0.00%)	10 (14.71%)	10	
HIV positive			0	0	0	
HPV vaccinated			0	0	0	
Total			15 (22.06%)	53 (77.94%)	68	

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The vast majority of cases, for which expedited treatment was chosen, were diagnosed with HSIL cytology (45 cases-66,17%), out of which 16 (36,36%) had a hrHPV positive test.

Another indication for excisional procedure without colposcopic biopsy was atypical squamous cells cannot exclude high grade (ASC-H) cytology with HPV positive result (23 cases-33,82%).

Only 39 (57,35%) out of 68 cases had a known HPV status.

Histopathological study indicated the presence of abnormal cellular proliferation, altered maturation and cytologic atypia in all examined lesions.

LSILs were observed in 15 cases (22,06%) and HSIL was diagnosed in 53 cases (77,94%).

LSIL were easily observed on low magnification, mostly because of the epithelial hyperplasia.

The most common feature seen in LSIL was nuclear atypia, characterized by nuclear enlargement with wrinkling nuclear membrane and hyperchromasia limited to the lower third of the epithelium.

Koilocytosis, (nuclear irregularities with a perinuclear cytoplasmic cavitation), which was also a LSIL mark, was recorded in 39 cases (57,35%).

Atypical mitosis was an inconspicuous finding among LSILs and always limited to the lower third of the epithelium.

In HSIL, nuclear atypia and cellular abnormalities were extended beyond the lower third of the epithelium, occupying the lower 2/3 seen HSIL (CIN 2) or the whole epithelial thickness, characteristic to HSIL (CIN 3).

HSIL (CIN 2) was identified in 31 of the cases (45,58%), while HSIL (CIN 3) was reported in 22 cases (32,35%).

HSIL (CIN 2) presented significant basal and parabasal nuclear atypia and abnormal mitotic figures that were confined to the lower 2/3 of the epithelium.

The nuclei of immature basal type cells were crowded, pleomorphic and enlarged.

Cytoplasm was scant and an increased nuclear: cytoplasmic ratio was observed.

In HSIL (CIN 3) immature basaloid-type cells and atypic mitosis were seen occupying the entire epithelial thickness.

Anisonucleosis, variation in nuclear size, was recorded in 3 cases (0,04%) (Figure 1).

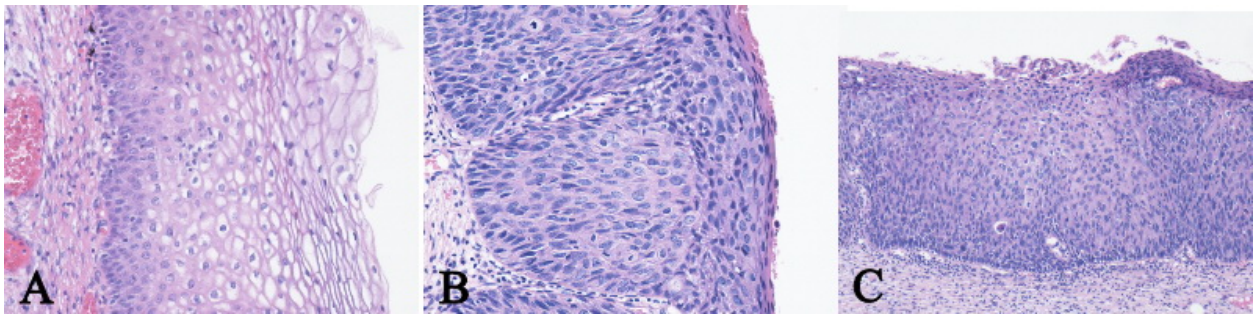

Figure 1 (A): Squamous epithelial hyperplasia demonstrating koilocytic atypia with nuclear abnormalities. Mitotic figures are confined to the lower third of the epithelium; LSIL, H&E stain, 40x. (B): Squamous epithelium showing altered architectural maturation with nuclear abnormalities occupying more than one third of the epithelial thickness, but presenting superficial cellular maturation. HSIL (CIN 2), H&E stain, 40x. (C): Squamous epithelium presenting cellular maturation loss on all its layers, increased nuclear: cytoplasmic ratio, with enlarged, hyperchromatic nuclei. HSIL (CIN 3), H&E stain, 20x.

We used immunohistochemical assessment (p16) for diagnosis confirmation in 24 cases (6 uncertain LSIL and 18 uncertain HSIL) that presented uncertain cytoplasmic differentiation located in the superficial or middle third of the epithelium.

p16 presented a block stain reaction at the epithelial level, on its entire thickness in HSIL cases, while LSIL was characterized by the absence of the stain, or a week reaction confined to the lower third of the epithelium.

Immunohistochemical analysis showed positive reaction for Mena in 48 of the investigated cases (70,58%).

Mena's immunoexpression at the epithelial level was recorded in 6(40%) out of 15 LSILs, 21(67,74%) out of 31 HSIL (CIN 2) and 21(95,45%) out of 22 HSIL (CIN 3), but we also found Mena positive cells located in the cervical stroma of 61(89,70%) samples.

In regard to the 24 uncertain cases Mena was positive in 1 case of LSIL and 18 cases of HSIL (100%).

The immunoreaction for Mena was identified with a predominantly cytoplasmic pattern in the cervical squamous epithelium and it mostly followed the nuclear abnormalities' pattern.

In all 6 LSIL cases, where Mena had a positive reaction, the immunopattern was confined to the lower third of the epithelium.

In HSIL (CIN 2), that presented a positive reaction to Mena, it was mostly limited to the lowest two thirds of the epithelial thickness (12 cases-57,14%) and in some cases (4 cases-19,04%) it was restricted to the lower third of the epithelium, while in other cases (3 cases-14,28%).

Mena stained the entire epithelial height, with a more intense reaction in the lower epithelial third.

We also observed an overall increase of the staining's intensity as the SILs' grade increased (Figure 2).

Mena's expression at the epithelial level in terms of intensity was statistically significant (ANOVA one-way test, $p<0.05$) (Figure 3).

As for the Mena staining area, we also observed a statistically significant pattern (ANOVA one-way test, $p<0.05$) (Figure 4).

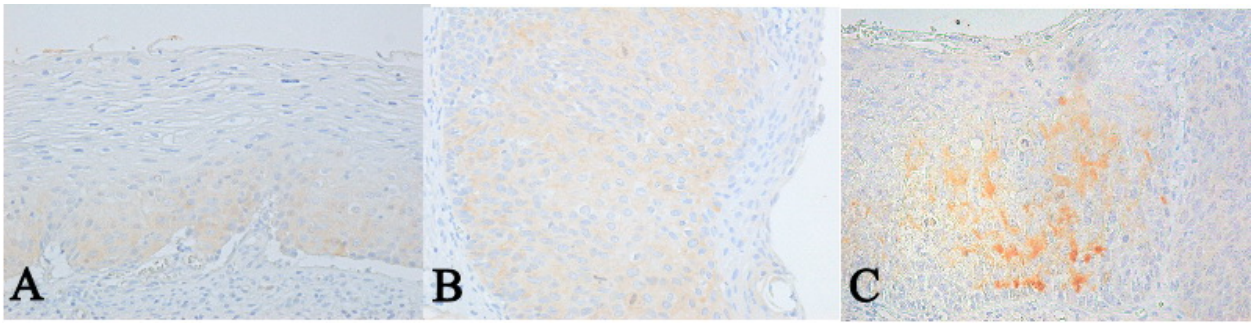

Figure 2. Mena immunoexpression in SILs: (A): LSIL, 40x; (B): HSIL (CIN 2), 40x; (C): HSIL (CIN 3), 40x.

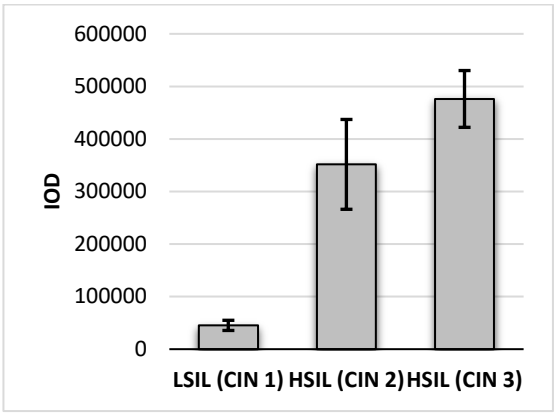

Figure 3. representation of Mena's expression in cervical squamous intraepithelial lesions in terms of intensity (IOD). A marked difference between Mena's staining in LSIL and HSIL is noticeable.

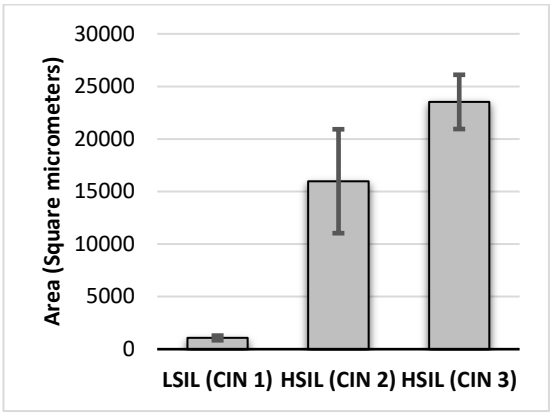

Figure 4. representation of Mena's expression in cervical squamous intraepithelial lesions in terms of area. A marked difference between Mena's staining in LSIL and HSIL is noticeable.

Regarding the stromal immunostaining pattern, we identified scatter Mena positive cells disposed usually in the vicinity of blood vessels, with a homogeneous distribution and a marked increased number in HSIL cases (Figure 5), compared with LSIL cases, a difference highly statistically significant (ANOVA test $p<0.05$) (Figure 6).

The staining presented a cytoplasmic, granular pattern that would correspond to a round-oval cell type that does not resemble a myocyte.

Given the staining used, we could not identify what origin those cells have.

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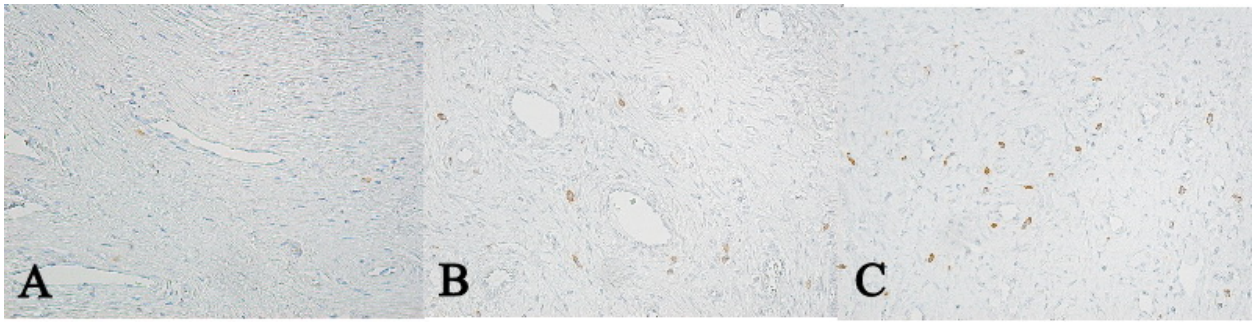

Figure 5. Mena immunoexpression in lesional cervical stroma A-C: (A): LSIL, 40x; (B): HSIL (CIN 2), 40x; (C): HSIL (CIN 3), 40x;)

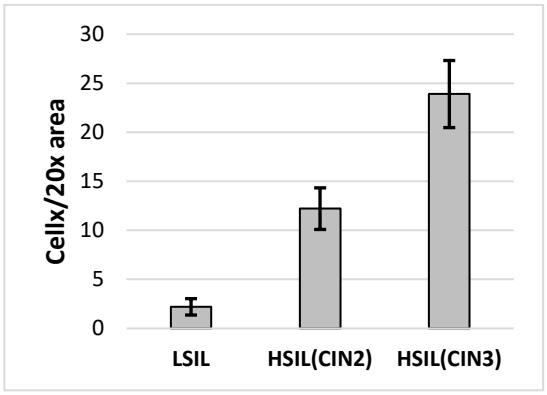

Figure 6. representation of the average cell number per field of view.

In order to evaluate the association between smoking and Mena's expression we used χ^2 test that demonstrated no statistically significance between them (p=0.658).

Discussion

According to 2020 GLOBOCAN estimates, cervical cancer ranks as the fourth most common diagnosed cancer in women throughout the world.

Even though it is a preventable disease [20], through mass vaccination and efficient screening programs, cervical cancer remains a significant public health problem in Romania, where it is the fourth leading cause of cancer mortality among females, being responsible for approximately 343 636 deaths in 2020 alone.

Virtually all cervical cancers and their precursors, are caused by HPV infection [21].

Among 200 HPV genotypes, 14 were designated as carcinogenic (high-risk) to humans (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and 12 types are low-risk, mainly related to genital warts (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP 6108).

HPV 16 and HPV 18 are associated with more than 70% of cervical cancers [22].

Fortunately, prophylactic HPV vaccines namely bivalent, quadrivalent and nonavalent vaccine became available more than 15 years ago [23] and vast studies proved their contribution to the HPV associated disease prevention.

In Romania, HPV vaccination may be administered, for free, to girls aged between 11 and 18, on parents' request.

However, the demand is quite low.

In our study group none of the patients were vaccinated against HPV infection.

Moreover, we could not asses the correlation between HPV status and other factors because HPV DNA was not tested in all cases.

Although HPV infection is a necessary condition to cancer development, it is not a sufficient one, as HPV related lesions may regress.

Most women [24] will become infected with a HPV type, but the majority of HPV infections are asymptomatic and they will clear within 2 years.

Risk factors that may prevent the natural clearance of HPV infection in some women have been extensively studied.

Tobacco smoking, long-term oral contraceptive use (>5 years) [25], IUD use, high parity and co-infection with human immunodeficiency virus (HIV) have been reported as risk factors in cervical oncogenesis.

However, recent studies' reports are conflicting [26] regarding them.

In our study group there was no correlation between COC use, parity, gravity or IUD use, except for a week association with smoking habit.

We used area of residence as a surrogate for the socioeconomic status and we did not find any statistically significant correlation and none of the patients were HIV positive.

Cervical intraepithelial neoplasia (CIN) is a precursor of cervical cancer caused by persistent HPV infection, mainly with the hrHPV subtypes [27].

CINs are graded into CIN 1, CIN 2 and CIN 3, based on the proportion of epithelial height occupied by basal-like, undifferentiated cells, which reflects the progressive loss of epithelial maturation.

In 2012, LAST [28] replaced the three-tiered CIN 1/2/3 nomenclature and adopted a two-tiered system, where condyloma and CIN 1 are included into the LSIL, and CIN 2, CIN 3 respectively, are assimilated by HSIL terminology.

The LSILs are self-limited, productive and transient viral infections, which display nuclear enlargement with irregular membrane, hyperchromasia and coarse chromatin, cytologic features termed „koilocytic atypia".

Mitotic figures may be present or not, but the epithelial squamous maturation is always retained.

Given the natural history of this affection [29], it usually does not require treatment, but observation management is much needed because approximately 15 % of these lesions persist and around 1% progress.

HSIL presents loss of the epithelial maturation (nuclear atypia and increased nuclear density) involving both the upper and the lower epithelial layers.

The atypical cells are immature, present high nuclear to cytoplasmic ratio, coarse chromatin and their polarity in the basal/parabasal layers is lost.

Mitosis generally involves the upper half of the epithelium and abnormal mitotic figures are not an unusual finding. CIN 2 and CIN 3 are subjectively distinguished by the degree of visible cell maturation.

In CIN 2 basaloid cells occupy the lower 2/3 of the epithelium with some degree of maturation or koilocytosis present on the epithelial surface, while in CIN 3 immature cells occupy the full thickness of the tissue [30].

HSILs are true cancer precursors and excisional treatment is highly recommended.

Nonetheless, their progression to invasive disease takes years and they may also regress, especially CIN 2.

As a result, treating young patients with excisional procedures for such conditions may be harmful, given the adverse pregnancy outcome associated with those procedures.

Therefore, an accurately diagnosis is much needed.

Immunohistochemical staining with p16 is proved to increase the diagnosis accuracy, but a high intra-and inter-observatory agreement has not been reached so far.

Furthermore, there is insufficient evidence that p16 positive lesions have a higher risk of progression.

In our study group, we identified 24 cases (35,29%) of morphological ambiguous cases, that were assessed with p16.

Using Mena staining we identified 1 out of 6 LSILs and 18 out 18 HSILs (100%).

In consequence, we consider that Mena staining may be of assistance in HSIL diagnosis.

Mena, a member of the Ena/VASP protein family, is an actin regulatory adhesion protein, that is defined at actinrich structures, like filopodia, focal adhesions, lamellipodia and cell-cell contacts, along with stress fibers.

It is encoded by ENAH gene found of chromosome 1. L.M. Chee et. all [31] demonstrated, using malignant keratinocytes derived from cutaneous squamous cell carcinoma, that Mena regulates actin-nuclear lamina associations, the architectural framework of the cell nucleus, chromatin remodeling and gene expression.

Another study [16] reported the up-regulation of Mena's expression in papillary thyroid cancer and it's correlation with tumor stage, local invasion, lymph node metastasis and clinical stage.

Another study [32] showed that homolog Mena acts as a transcriptional coactivator of the EGFR signaling pathway and modulates considerably the growth of pancreatic malignant cell lines related to the EGFR signaling.

Moreover, the authors reported that a Mena isoform, $hMena^{+11a,}$ which is a splice variant of hMena, that has an epithelial phenotype and was isolated from a breast cancer cell line is expressed selectively in cancer cells which are using the EGFR to drive proliferation.

In a more recent study [15], Kunpeng Hu et. al concluded that Mena is upregulated in hepatocellular carcinoma and correlated it with tumor differentiation and stage.

In addition, they identified Mena as an independent prognostic factor for hepatocellular carcinoma patients, based on the association between Mena's overexpression and poor prognosis.

Notably, Sijia Na et. al observed [18] that an elevated expression of Mena in oral squamous cell carcinoma.

They also reported that the overexpression of Mena was associated with lymphatic metastasis and TMN stage.

Additionally, they shoved that Mena's expression was correlated with the expression of

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epithelial mesenchymal transition markers (E-cadherin and Vimentin), but did not find a statistically relevant association with the expression of proliferation marker Ki-67.

Moreover, they reported a correlation between the expression of Mena and MMP-2, which is a tumor invasive marker and also between Mena's expression and poor prognosis.

A recent study [10] that evaluated different classes of actin-binding proteins in clear-cell renal cell carcinoma revealed that Mena is strongly up-regulated in tumor associated vascular endothelial cells (VEC) compared to normal adjacent tissue VEC and it proved coordinated up-regulation of PNF1 (Profilin 1) and Mena in tumor associated vascular endothelial cells in situ.

Dan-Dan Wang et. all [14] analyzed the ENAH expression (Mena) in gastric cancer and found a significant correlation between it and tumor size, depth of tumor infiltration, TMN stage and local lymph node metastasis.

Interestingly, no correlation was found between distant metastasis and Mena's expression, but the 5-year survival rate of patients with overexpressed Mena was significantly lower than the overall survival rates of patients that presented a low Mena expression.

However, a recent study showed that Mena overexpressing cells are directly implicated in the metastasis process [33].

Mena-expressing cells are found in tumor microenvironment of metastasis (TMEM), in contact with an endothelial cell and a macrophage forming one complex structure.

Intravasation in mouse breast carcinoma occurs at TMEM's level, and its density is correlated with an increased risk of distant metastasis in ER-/HER2+breast carcinoma.

Mena's expression in breast cancer was firstly reported many years ago [34].

Since then, more studies proved that normal acinar and ductal breast epithelium do not stain with Mena, whereas benign breast lesions express rarely and weekly Mena phenotype [12].

Also, Di Modugno et. al, observed that positivity increased progressively in benign breast lesions that possess a high risk of malignant transformation and concluded that

Mena's overexpression occurs early in breast carcinogenesis.

Regarding Mena's expression in invasive breast cancer, a significantly statistical correlation was found with tumor size, proliferation index and other prognostic factors like HER-2 overexpression.

Another study [35] showed that Mena's deficiency in a PyMT trans genetic model of breast cancer reduces significantly tumor cell motility, intravasation and metastasis.

We found only one report regarding Mena's expression in cervical cancer and it's precursors [19] that described an immunoexpression in all studied cases, with various degrees of intensity.

In our study group, Mena was expressed in 48 out of 68 cases at the cervical epithelial lesions (70,58%), staining 6(40%) out of 15 LSILs, 21 (67,74%) out of 31 HSIL (CIN 2) and 21(95,45%) out of 22 HSIL (CIN 3).

We believe that larger studies are required in order for an accurate assessment of Mena's expression on cervical precancers to be made, as both studies were performed on a rather small number of cases (30 and 68 respectively).

However, our finding regarding Mena's immunoexpresion on LSILs may be in accordance with other studies [12] that report Mena's staining on benign breast lesions as inconstant, with an increased positivity related to the malignant transformation potential.

The same study [19] reported Mena's expression to increase as the cervical lesion's grade increased.

In our study, the intensity of Mena's expression at the epithelial level was statistically significant (ANOVA one-way test, $p<0.05$) and it was considerably higher in HSILs, compared to LSILs.

As for the Mena staining area, we also observed a statistically significant pattern (ANOVA one-way test, $p<0.05$), all of this suggesting that Mena stains especially abnormal cells and that its expression intensity correlates with the risk of malignant transformation.

In addition, Gurzu S. et all reported no stromal immunoexpression of Mena.

In contrast, in our study group, we observed Mena positive cells located in the cervical stroma of 54 (79,41%) samples. Moreover, the number of this cells was significantly higher in HSILs, compared with LSILs ($p<0.05$).

The staining presented a cytoplasmic, granular pattern that would not correspond to the shape of a myocyte.

Given the staining used, we could not identify the type or the origin of those cells.

Some studies [14] performed on various types of cancer described a population of Mena expressing cells that are intimately [33] linked to the invasion and metastasis process.

Whether it is the same cell population or not, remains to be determined, as well as their function.

Conclusion

Our study failed to identify a correlation between SILs and risk factors such as combined oral contraceptive use, IUD use, parity, gravity, except for the tobacco smoking habit that proved to be related to the cervical lesions' development.

Mena was expressed in most of the analyzed SILs and its expression was significantly correlated with lesions' grade in terms of both intensity and area.

Further studies are needed to validate Mena as an early stage of cervical carcinogenesis marker.

Conflict of interests

None to declare

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