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Original article

Cytological and Bacteriological Assessment of the Cervix in Cases Having Nabothian Cysts

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Abstract:

Background: Nabothian cysts are common benign cervical lesions often discovered incidentally during routine gynecological examinations. While they are typically asymptomatic, they may occasionally present with various clinical complaints. This study aimed to correlate between cytological or bacteriological findings and presence of nabothian cysts. **Methods:** This prospective observational study was carried out on one hundred women with confirmed cervical Nabothian cysts. The study was conducted at the Obstetrics and Gynecology Department of Benha University Hospitals between April 2022 and December 2022. Detailed clinical evaluations, including history taking, transvaginal ultrasound examination, cytological diagnosis using the Papanicolaou method, and bacteriological examination of cervical swabs and cyst aspirates, were performed. Results were analyzed to assess the characteristics of Nabothian cysts, cytological findings, and microbiological assessments. **Results:** Of the 100 participants, 60% were in the 31-40 age range, with 37% having two children. Complaints included 63% reporting discharge, 51% experiencing pelvic pain, and 8% facing infertility issues. Most cysts (68%) were larger than 10mm and located in the upper part of the cervix (57%). Cytological results revealed 8% with intraepithelial lesions and 3% with malignancy. Bacteriological assessments showed 40% had bacterial growth, with 28% displaying gram- negative bacilli. The presence of bacterial growth correlated with inflammatory changes and malignancy. **Conclusion:** Cytological and bacteriological assessments of Nabothian cysts in women with related complaints can provide valuable insights into their etiology and associated conditions. The presence of bacterial growth and specific bacterial strains may be linked to inflammatory changes and malignancy in these cysts.

Keywords: Nabothian Cysts; Cytological and bacteriological cervical lesions; Transvaginal Ultrasound.

Introduction

Nabothian cysts are common gynecological conditions in reproductive women. They are generally multiple, translucent or opaque, and whitish to yellow. Nabothian cysts usually occur at the transformation zone of the uterine cervix and are a few millimeters to 3–4 cm in diameter [(1)].

It is a chronic inflammation of the cervix due to interstitial or epithelial squamous metaplasia, which obstructs the orifice of the gland, causing the endocervical glands to dilate cystically and the cervix to expand [(2)].

The presence of multiple nabothian cysts can be related to some pathological conditions, mostly as a consequence of an inflammatory reaction as chronic cervicitis due to microbial causes, such as Chlamydia, Streptococcus, and Staphylococcus and granulomatous diseases such as pulmonary tuberculosis [(3)].

Nabothian cysts can grow in the cervix and is usually small and asymptomatic. Large and extensive cysts are located deeper in the cervix. The nabothian cyst tunnel cluster is observed as a result of multicystic dilatation of the endocervical glands [(4)].

Nabothian cysts rarely cause any problems and need no treatment. However, if they are associated with chronic cervicitis (which is likely to cause blockage of ducts), they can be drained when cervicitis is treated [(5)].

In cervical smear and biopsy samples they are generally considered as benign structures. It is difficult to distinguish them from a minimal-deviation adenocarcinoma, which is classified as a special type of cervical adenocarcinoma [(6)].

Nabothian cysts (also called mucinous retention cysts or epithelial cysts) are frequent at the squamocolumnar junction, an anatomical point of the cervix that is the target of brush sampling during cervical screening cytology [(7)].

Inspissated mucus contained inside these cysts may exhibit a granular character or can be filled with granulocyte fragments, resembling tumor diathesis, a key cytological feature of frankly invasive carcinoma. Moreover, necrotic debris in glands colonized by high-grade squamous intraepithelial lesions, may also mimic tumor diathesis [(8)].

The aim of the work was to correlate between cytological or bacteriological findings and presence of Nabothian cysts.

Patients and methods

This prospective observational study was conducted at the Obstetrics and Gynecology Department of Benha University Hospitals from April 2022 to December 2022. It involved the enrollment of 100 women from the outpatient clinic who had confirmed cervical nabothian cysts.

An informed written consent was obtained from the patients. Every parent received an explanation of the purpose of the study and had a secret code number. The study was done after being approved by the Department of the Research Ethics Committee, Faculty of Medicine, Obstetrics and Gynecology Department, Benha University.

Inclusion criteria were women in childbearing period (18-44ys), women presented with any of the following complaints: Persistent vaginal discharge, Chronic pelvic pain, or Primary or secondary unexplained infertility and found to have nabothian cyst on transvaginal ultrasound or during clinical examination.

Exclusion criteria were other types of cervical cysts, nonconsenting patients and cases with previous LEEP or conization.

Methods:

All patients were subjected to the followings:

Full history including Personal data: (Name, age, address, phone number, occupation and parity). The patients were clinically evaluated according to their

complaint: A) Persistent vaginal discharge was evaluated for type, color, frequency and history of previous treatment. B) Pelvic pain patients were evaluated for nature of pain, relation to intercourse, correlation to the cycle, and history of previous investigations and treatment. C) Unexplained infertility was evaluated for type (1ry or 2ry), previous full investigation, and previous treatment. Past history: (medical history and surgical history).

Transvaginal ultrasound:

Each participant underwent one transvaginal ultrasound exam [9] to determine the site, size, and number of cervical nabothian cysts. All ultrasound examinations were performed by Voluson or Mindray DC70 expert machine with a 6-10 MHZ endocavitary probe according to the following steps (Figure 1): 1) prior to the transvaginal ultrasound, participants were positioned in lithotomy, and the urinary bladder was emptied, with the vaginal canal free from tampons. 2) The transducer was inserted through the vagina, and, when possible, patients were allowed to self-insert the transducer. 3) The transducer was prepared before commencing the examination. 4) A gel was applied to the top of the transducer and covered with a condom. 5) The examination included an assessment of the size, shape, and position of the uterus, cervix, and vagina. 6) Evaluation of the right and left adnexa was also performed.

Figure 1: Transvaginal ultrasound view of a round two-chamber cystic mass on the cervix.

Cytological diagnosis:

Cytological diagnostics was carried out using the Papanicolaou method (Pap test). When evaluating the results of a cytological study, we used the Papanicolaou classification, the most common classification of pathological examination of cervical smears, which has 5 types [10]: Type I -no atypical cells, normal cytological picture. Type II-a slight change in cellular elements due to the inflammatory process, manifested by a slight increase in the nucleus, the appearance of cells of metaplastic epithelium. Type III -there are single cells with changes in the ratio of the nucleus and cytoplasm, dyskaryosis, the diagnosis is not clear enough, a repeat cytological examination is required, or a histological examination is necessary. Type IV-individual cells are found with signs of malignancy, namely with enlarged nuclei and basophilic cytoplasm, uneven distribution of chromatin. Type V-there are numerous atypical cells in the smear.

Bacteriological examination:

Bacteriological examination of the cervical swabs and aspirated fluid from the cysts under colposcopic or US guidance, and cytological examination of cysts' aspirates as follow; the cervical swabs where the patients were asked to insert the swab into the vagina approximately 4 to 5 cm and

then to rotate it several times before placing it into a capped tube [11].

They were also given a plastic container and asked to collect 25 to 30 ml of first-void urine. The first specimen collected was the self-obtained vaginal swab [9].

Vaginal specimens were then collected for diagnosis of vulvovaginitis, if indicated. The last specimen collected was for Pap smear, if indicated at the time of this examination.

The plates were kept under microaerophilic conditions at 37 ˚C. Liquid media were examined daily for 10 days for the color change indicating growth.

All of the participating laboratories have had considerable experience doing cultures, and each laboratory performed cultures using its standard procedure.

Isolation attempts were performed on swabs (processed separately) obtained from the cervix.

Approval code: MS -6-9-2022

Statistical analysis

Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences) version 28 (IBM Inc., Chicago, IL, USA). Kolmogorov Smirnov normality test and histograms were used to test the distribution of quantitative variables to select accordingly the type of statistical testing: parametric or nonparametric. Normally distributed quantitative variables (e.g., age) were expressed as mean and standard deviation (SD). Non-normally distributed quantitative variables were expressed as median and interquartile range (IQR). Categorial variables (e.g., sex) were expressed as frequency and percentage.

Results

Out of the initial 190 women, 90 were excluded from the study for various reasons, including 25 who did not have nabothian cysts, 10 with prior gynaecological operations, and 25 who declined to participate. As a result, the final study cohort consisted of 100 women who were randomized and included in the research. Figure 2

Regarding the age distribution of the study group, with the majority falling in the 31-40 years age range (60%), followed by those over 40 years (28%), and the 18-30 years age group (12%). Regarding parity: 8 (8%) of the cases had no children (parity 0). 18 (18%) of the participants had one child (parity 1). The majority, with 37 (37%) cases, had two children (parity 2). 28 (28%) participants had three children (parity 3). 9 (9%) of the cases reported having more than three children (parity more). Regarding the distribution of medical history within the study group of women with Nabothian cysts. 43 (43%) participants had a history of Diabetes Mellitus (DM). 33 (33%) participants had a history of Hypertension (HTN). Regarding the distribution of complaints within the study group of women with Nabothian cysts. 63 (63%) participants reported complaints related to Discharge. 51 (51%) participants reported complaints related to Pelvic pain. 8 (8%) participants reported complaints related to Infertility. Table 1

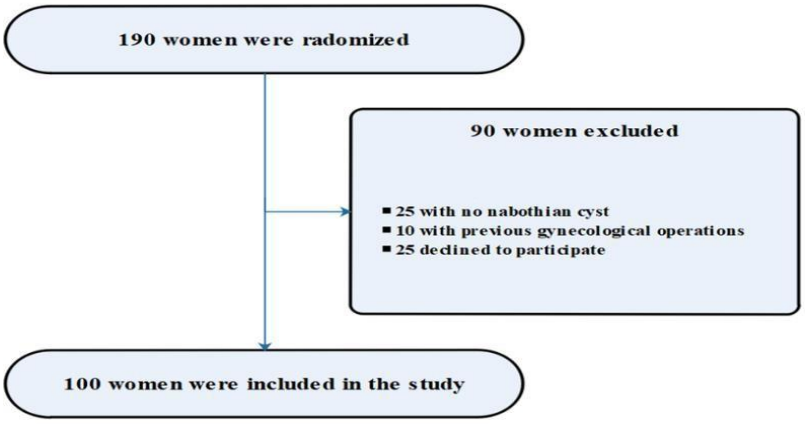

Figure 2. Flowchart of the studied patients

Table 1: Study group regarding age, parity and medical history.

Age	**No.**	**%**
18-30 Y	12	12
31-40 Y	60	60
40-44 Y	28	28
Age (mean ± SD)	32.3	10.4
Parity	No.	%
0	8	8
1	18	18
2	37	37
3	28	28
More	9	9
Medical history *	No.	%
DM	43	43
HTN	33	33

*One case may have more than disease.

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Regarding the characteristics of cervical Nabothian cysts within the study group: In terms of the size: 32 (32%) participants had cysts smaller than 10 mm. 68 (68%) participants had cysts larger than 10 mm. In terms of the site: 57 (57%) participants had cysts located in the upper part of the cervix. 43 (43%) participants had cysts located in the lower part of the cervix. In terms of the number: 71 (71%) participants had a single Nabothian cyst. 29 (29%) participants had multiple Nabothian cysts. Figure 3

In terms of cytological examination: 8 participants had Intraepithelial Lesions, accounting for 8% of the total study population. Three participants were diagnosed with Malignancy, making up 3% of the study group. In terms of Inflammatory Changes: 52 participants had Mild inflammatory changes (52% of the study population). 20 participants had Moderate inflammatory changes (20% of the study group). 28 participants had Marked inflammatory changes (28% of the study population). 28 (28%) of the participants showed Squamous Metaplasia. No participants underwent HPV-co-testing. Table 2

The presence of bacterial growth was shown in 40 (40%) of the participants, which accounts for of the total study population. While 60 (60%) of the participants had no bacterial growth. Regarding the microbiological assessment: 28 (28%) of the participants showed the presence of Gram-negative bacilli. 4 (4%) of the participants had Gram-positive bacilli. 16 (16%) of the participants had Gram-positive cocci. Table 3

Table 2: Study group regarding cytological examination.

		No.	%
Intraepithelial lesion		8	8
Malignancy	Squamous cell carcinoma	1	1
	Adenocarcinoma	2	2
Inflammatory changes	Mild	52	52
	Moderate	20	20
	Marked	28	28
Squamous metaplasia		28	28
HPV-co-testing		0	0

Table 3: Study group regarding Bacteriological examination and Microbiological assessment.

Bacteriological examination		No.	%
Growth	Present	40	40
	Absent	60	60
Microbiological assessment *		No.	%
g - ve bacilli		28	28
g + ve bacilli		4	4
g + ve cocci		16	16

*One case may have more than one organism.

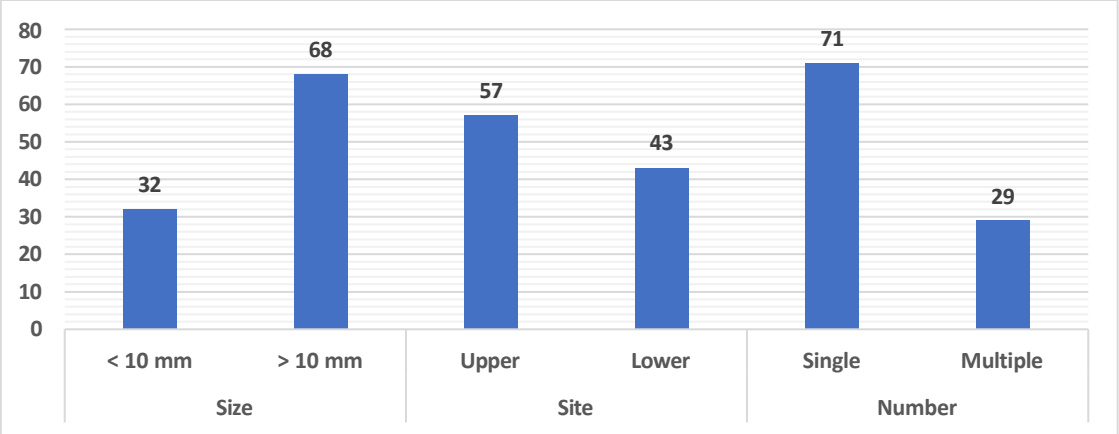

Figure 3: Study group regarding cervical Na.

In the "Mild" category of inflammatory changes, there were 11 cases (39.3%) with gram-negative bacilli, 3 cases (75.0%) with gram-positive bacilli, 7 cases (46.7%) with gram-positive cocci, and 31 cases (58.5%) with no bacterial growth. In the "Moderate" category, there were 4 cases (14.3%) with gram-negative bacilli, 1 case (25.0%) with gram-positive bacilli, 3 cases (20.0%) with gram-positive cocci, and 12 cases (22.6%) with no bacterial growth. In the "Marked" category, there were 13 cases (46.4%) with gram-negative bacilli, 0 cases (0.0%) with gram-positive bacilli, 5 cases (33.3%) with gram-positive cocci, and 10 cases (18.9%) with no bacterial growth. Table 4

Under the "No Malignancy" category, there were 27 cases (96.4%) with gram-negative bacilli, 4 cases (100.0%) with gram-positive bacilli, 14 cases (93.3%) with gram-positive cocci, and 52 cases (98.1%) with no bacterial growth. In the "Malignancy" category, there were 1 case (3.6%) with gram-negative bacilli, 0 cases (0.0%) with gram-positive bacilli, 1 case (6.7%) with gram-positive cocci, and 1 case (1.9%) with no bacterial growth. Table 5

In the "Intraepithelial" category, there were 1 case (3.6%) with gram-negative bacilli, 0 cases (0.0%) with gram-positive bacilli, 1 case (6.7%) with gram-positive cocci, and 6 cases (11.3%) with no bacterial growth. In the "Squamous Metaplasia" category, there were 9 cases (32.1%) with gram-negative bacilli, 3 cases (75.0%) with gram-positive bacilli, 5 cases (33.3%) with gram-positive cocci, and 11 cases (20.8%) with no bacterial growth. Table 6

Table 4. Association between microbiological assessment and inflammatory changes.

		G- ve bacilli		G + ve bacilli		G + ve cocci		No growth		Test	p
		N	%	N	%	N	%	N	%		
Inflammatory changes	**Mild**	11	39.3%	3	75.0%	7	46.7%	31	58.5%	3.537	0.469
	Moderate	4	14.3%	1	25.0%	3	20.0%	12	22.6%	1.135	0.766
	Marked	13	46.4%	0	0.0%	5	33.3%	10	18.9%	11.405	0.323

Table 5. Association between microbiological assessment and malignancy.

		g - ve bacilli		g + ve bacilli		g + ve cocci		No growth		Test	p
		N	%	N	%	N	%	N	%		
Malignancy	**No malignancy**	27	96.4%	4	100.0%	14	93.3%	52	98.1%	0.453	0.928
	Malignancy	1	3.6%	0	0.0%	1	6.7%	1	1.9%	0.056	0.812

Table 6: Association between microbiological assessment and intraepithelial lesion and squamous metaplasia.

	g - ve bacilli		g + ve bacilli		g + ve cocci		No growth		Test	p
	N	%	N	%	N	%	N	%		
Intraepithelial	1	3.6%	0	0.0%	1	6.7%	6	11.3%	1.480	0.682
Squamous metaplasia	9	32.1%	3	75.0%	5	33.3%	11	20.8%	3.197	0.364

Discussion

Nabothian cysts, also known as mucinous retention cysts, commonly occur at the squamocolumnar junction of the cervix and can mimic features resembling tumor diathesis [(5)]. These benign lesions primarily affect women of reproductive age, forming through the interaction of squamous and columnar epithelia, trapping mucoid material from the endocervical glands [(12)]. Typically, they manifest as small, asymptomatic bumps on the cervical surface and resolve spontaneously [(7)]. This study, conducted on 100 women with confirmed cervical Nabothian cysts, aimed to correlate cytological and bacteriological findings with these cysts, representing the first attempt to establish such a connection. Comprehensive clinical evaluations, including transvaginal ultrasound, cytological diagnosis, and bacteriological examination, were performed at Benha University Hospitals from April 2022 to December 2022. While rare case reports describe treatment for large, symptomatic Nabothian cysts, this research contributes to our understanding of these cysts' characteristics and potential diagnostic implications, emphasizing the importance of avoiding unnecessary hysterectomies.

Regarding the age distribution of the study group, with the majority falling in the 41-50 years age range (60%), followed by those over 50 years (28%), and the 31-40 years age group (12%). Regarding parity: 8 (8%) of the cases had no children (parity 0). 18 (18%) of the participants had hysterectomies. [y 1). The majority, with 37 (37%) cases, had two children (parity 2). 28 (28%) participants had three children (parity 3). 9 (9%) of the cases reported having more than three children (parity more). Regarding the distribution of medical history within the study group of women with Nabothian cysts. 43 (43%) participants had a history of Diabetes Mellitus (DM). 33 (33%) participants had a history of Hypertension (HTN).

Pelvic examinations of a 46-year-old woman (gravida 3, para 2) was admitted to our gynaecology clinic. She reported that an ultrasonography assessment performed 5 years earlier revealed a cervical cyst with a 3-cm diameter. revealed a uterus with normal bilateral adnexa, but the size was equivalent to that of a uterus during the 12th week of pregnancy [(1)].

The enlarged cervix was palpable during a rectovaginal examination. Transvaginal ultrasonography confirmed that the uterus was abnormally large and showed that it had a heterogeneous myometrial echotexture and an unremarkable endometrium with a 5 cm× 4.5 cm anechoic cyst in the posterior cervical wall. Pelvic computed tomography revealed an enlarged and distended bladder and bilaterally dilated ureters and confirmed the presence of a cervical cyst with a 5-cm diameter [(1)].

Regarding the distribution of complaints within the study group of women with nabothian cysts. Vaginal discharge can be a common symptom associated with various gynaecological conditions, including cervical cysts. In the context of Nabothian cysts, the presence of mucus-filled cysts on the cervix can disrupt the normal cervical secretions, leading to an increase in vaginal discharge [(13)].

The high percentage (51%) of participants reporting pelvic pain as a complaint indicates that this symptom is also quite common among women with nabothian cysts. Pelvic pain can have various causes, and in the case of Nabothian cysts, it may be related to the size and location of the cysts. Larger or symptomatic cysts can exert pressure on surrounding tissues, leading to discomfort or pain [(4, 14)].

Regarding the characteristics of cervical Nabothian cysts within the study group: In terms of the size: 32 (32%) participants had cysts smaller than 10 mm. 68 (68%) participants had cysts larger than 10 mm. In terms of the site: 57 (57%) participants had cysts located in the upper part of the cervix. 43 (43%) participants had cysts located in the lower part of the cervix. In terms of the number: 71 (71%) participants had a single Nabothian cyst. 29 (29%) participants had multiple Nabothian cysts. Nabothian cysts generally being small-sized and multiple are common gynaecopathological conditions of women in reproductive age. [(15)]

In terms of cytological examination: 8 participants had Intraepithelial Lesions, accounting for 8% of the total study population. 8 participants were diagnosed with Malignancy, making up 8% of the study group. In terms of Inflammatory Changes: 52 participants had Mild inflammatory changes (52% of the study population). 20 participants had Moderate inflammatory changes (20% of the study group). 28 participants had Marked inflammatory changes (28% of the study population). 28 (28%) of the participants showed Squamous Metaplasia. No participants underwent HPV-co-testing.

High standards of cytology have to be maintained and a low false-positive rate is essential, as positive cytology results cause anxiety and possibly unnecessary colposcopy examinations. Different quality control measures are suggested for cervical cytology, one of which is cytohistological correlation of positive and/or discordant cases [(8)].

A 32-year-old woman had been followed for several years due to a history of cervical dysplasia. On the last routine Pap test smear, atypical squamous cells consistent with H-SIL were identified along with TD-like material, thus suggesting invasive carcinoma. On cervical biopsies, areas containing typical low-grade squamous intraepithelial lesion (L-SIL) and H-SIL were diagnosed, but no invasive carcinoma [(8)].

The subsequent conization showed cellular morphological alterations consistent with koilocytosis associated with H-SIL, and H-SIL colonizing cervical glands, next to abundant Nabothian cysts. Histological examination of the entire conization specimen showed 3 foci of H-SIL

colonizing cervical glands, Figure 1C (square and inset) next to abundant Nabothian cysts. Some of these were filled with inspissated mucus, containing neutrophils and necrotic-like material, features reminiscent of the cytological finding of TD [8].

Cervical nabothian follicles is commonly benign but we should keep in mind the rare adenoma malignum on imaging and histopathology. Multilocular cystic lesions in the uterine cervix can vary widely from common benign lesions to malignant lesions rare [4].

The presence of bacterial growth was shown in 40 (40%) of the participants, which accounts for of the total study population. While 60 (60%) of the participants had no bacterial growth.

Regarding the microbiological assessment: 28 (28%) of the participants showed the presence of Gram-negative bacilli. 4 (4%) of the participants had Gram-positive bacilli. 16 (16%) of the participants had Gram-positive cocci.

In the case report shows that large, deep nabothian cysts can cause CUR in women. Therefore, although large nabothian cysts are rare, they should be considered as potential causes of pelvic masses and urinary symptoms in women. Nabothian cysts can be distinguished from other mucus-producing cervical malignancies on the basis of symptomatology, preoperative examination results, and imaging techniques such as magnetic resonance imaging [1].

The novelty of the current study is multifaceted. Firstly, it seeks to establish a correlation between cytological and bacteriological findings and the presence of Nabothian cysts, a relationship that remains relatively unexplored in existing literature.

Furthermore, our study adopted a comprehensive approach by concurrently assessing both cytological and bacteriological aspects, allowing for a more understanding of how nabothian cysts may impact cervical health. This study also brings clinical relevance by examining the symptoms and complaints associated with Nabothian cysts, including discharge, pelvic pain, and infertility, thus bridging the gap between laboratory findings and real-world patient experiences.

Lastly, the identification of diverse bacterial types within the cervical area of women with nabothian cysts, such as Gram-negative bacilli, Gram-positive bacilli, and Gram-positive cocci, contributes a novel microbiological dimension to the research, potentially influencing diagnostic and therapeutic strategies while advancing our knowledge of cervical health.

Conclusion

In this prospective observational study, we have provided valuable insights into the clinical significance of Nabothian cysts in women of childbearing age. Our findings reveal the diverse clinical presentations associated with these cysts, including persistent vaginal discharge, chronic pelvic pain, and unexplained infertility. Of note, cytological assessments identified intraepithelial lesions and malignancy in a subset of cases, showing the importance of regular cervical screening in this population.

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Author contribution

Authors contributed equally in the study**.**

Conflicts of interest

No conflicts of interest

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