Frequency of cervical intraepithelial neoplasia and infectious agents for vaginitis in menstrual cycle phase

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Summary

Objective: The study objective was to verify differences in the diagnosis of infectious agents and CIN in cytological smears in the proliferative and secretory phases of the menstrual cycle.

Methods: A retrospective study was carried out at the Federal University of "Triângulo Mineiro". Presence of *Candida albicans, Trichomonas vaginalis,* clue cells, Doderlein bacilli, cytolytic flora, coccoid bacillus, CIN and HPV were collected from the vaginal cytology tests, cervical and endocervical in healthy women of reproductive age from 1994 to 2004 (about 14,000 in total). The cytologies were divided into two groups: proliferative and secretory phase. Chi-square and Fisher's exact tests were used for statistical analysis with the significance level set at less than 0.05.

Results: The frequency of cytolysis and candidiasis was higher in the secretory phase of the menstrual cycle ($p < 0.0001$). When the presence of CIN associated with vulvovaginitis was evaluated, there was no significant difference in cytologies with CIN between the first and the second phases of the menstrual cycle.

Conclusion: Frequency of the cytolytic flora and *Candida albicans* is influenced by the phase of the menstrual cycle, but CIN is not.

Key words: Vaginitis; Lactobacillus; Candida; Bacterial vaginosis; Papanicolaou smear; CIN.

Introduction

Vaginal infections may be related to pH alteration, and differences among women in the proliferative and secretory phases of the menstrual cycle may occur. The ability of lactobacilli has been greatly recognized recently because of its protection against vaginal infections by the production of lactic acid, altering pH [1-5]. When a lactobacilli imbalance occurs, mainly the ones which produce H202, vulvovaginitis [5,6], recurrent infections of the urinary tract [2] and an augmented of risk of premature delivery [7, 8] can occur.

A large number of pathogenic and non-pathogenic organisms may be observed in vaginal microflora [9]. Diverse studies performed with the objective of establishing the frequency of the most common infectious agents for vaginitis have shown varying results. The prevalence found for *Gardnerella vaginalis* varied between 8% and 75%, *Candida albicans* presented 2.2% and 30%, and *Trichomonas vaginalis* between zero and 34% [10-16]. Moreover, infectious agents for vaginitis in Papanicolaou findings and vaginal pH are associated with women's age [17].

For a correct diagnosis of bacterial vaginosis, three of four criteria must be fulfilled: 1) typical homogeneous discharge, 2) positive sniff test, 3) vaginal pH more than 4.5, and 4) presence of *clue cells* in Papanicolaou smears [18]. Bacterial vaginosis is considered a risk factor for gynecologic and obstetric consequences, such as premature rupture of membranes, premature labor and delivery, fetal loss, low birth weight, postpartum endometritis, and pelvic inflanmmatory disease. [19]

The role of BV in the CIN genesis has been the focus of many current investigations. A possible correlation between BV and CIN was postulated in the 1970's [20]. A possible link between BV and CIN was also studied by Platz-Christensen, relating CINIII/carcinoma *in situ* with BV [21].

Murta *et al.* demonstrated that there is an association between *clue cells* and infection by human papillomavirus (HPV) [9]. Some factors, which can alter the vaginal microenvironment, such as vaginal hygiene practices [22], bacterial vaginosis [23], and sexually transmited diseases [24] have been proposed as co-factors for the persistence of HPV, and, therefore, could have a role in cervical carcinogenesis. As vaginal pH results from an interposition of multiple factors modifying this vaginal microenvironment, in that it is possible to measure it in a cheap and easy way, it could be used as a marker in epidemiologic studies and in the clinical practice of cervical carcinogenesis.

A mechanism proposed for the association of BV and CIN is that carcinogenic nitrosamines act independently or together with HPV [25]. Nitrosamines are substances resulting from nitrosation of amines, a process which can be catalized by bacterial enzymes. Thus, a combination of amines, nitrites, nitrate and bacteria, such as the ones found in the vagina in BV, may act in the cervix independently or associated with other agents, such as viruses [26, 27].

Vaginal candidiasis can have some exogenous predisposing factors, such as the use of antibiotics, oral contraceptives, gestation, hormone replacement therapy and

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unbalanced diabetes mellitus [28, 29]. In the absence of these factors, clinical observations show that vaginal candidiasis occurs more frequently during the luteal phase of the menstrual cycle, in which the blood levels of estrogen and progesterone are higher, and vaginal pH is decreased [30]. Hysterectomy may influence the frequency of cytological finding of *Candida sp* in women over 60 years old [31]. On the contrary, women in premenarche and postmenopause, who are not receiving hormone replacement therapy, rarely present it [29].

The aim of the study was to verify differences in the diagnosis of infectious agents and CIN in cytological smears of women in reproductive age, in the proliferative and secretory phases of the menstrual cycle. With that, it could be possible to know if the acid or neutral pH could contribute to the prophylactic treatment of vaginal infections. It is known that the secretory phase (O), of the menstrual cycle is more acidic than a proliferative phase.

Material and Methods

Patients

A retrospective study was carried out at the Cytopathology Department of the University Federal "Triângulo Mineiro" (UFTM). Samples of *Candida albicans, Trichomonas vaginalis, clue cells,* Doderlein bacilli, cytolytic flora, coccoid bacillus, CIN I, CIN II, CIN III and HPV were collected from vaginal cytology tests, the cervix and endocervix in healthy women of reproductive age, seen in the Outpatient Service of the Department of Gynecology and Obstetrics (UFTM), from 1994 to 2004, during a routine appointment (about 14,000 in total). Endocervical samples (brush), and cervical and vaginal samples (spatula) were obtained from non-pregnant women in reproductive age who were not undergoing hormonal therapy, douches or vaginal medication. Patients were seen in the gynecological position and the uterine cervix was exposed with a sterile and non-lubricated speculum. Smears were fixed in ethyl alcohol and obtained using the Papanicolaou method. Information was obtained by experts, trained by cytopathologists, who initiated the use of the Papanicolau test in our institution, using the same diagnostic criterion related to *Trichomonas vaginalis,* Candida sp, *clue cells,* Doderlein bacillus and vaginal flora. The intention was to divide the cytologies into two groups according to the phase of the menstrual cycle: proliferative and secretory phase, collected, respectively, from the 8th-12th and 18th-22nd days.

Cytological criteria [10, 32-34]

Clue cells are squamous cells covered with coccobacilli whose cytoplasmatic borders present as smudged. Candida sp was diagnosed when pseudo-hyphae were seen, weakly stained with eosin or sometimes with hematoxylin, and/or small spores (diameters of 2-4 mm), stained pale pink. *Trichomonas vaginalis* was diagnosed when a unicellular organism of ovoid or rounded shape was viewed (diameter of 8-20 mm), with pallid or grayish cytoplasm. It could have eosinophilic granules at its center and a vesicular or crescent-shaped nucleus, lightly stained by hematoxylin. Cytolysis was defined as a pale staining, vesicular nuclei with little or no cytoplasm of the intermediate cells predominate in such smears. Coccobacilli are characterised by bacilli and cocci organisms diffusely scattered, may occur in clumps and as microcolonies. Lactobacilli are characterised by presence of the elongated bacillary structures.

CIN and HPV

The cytological smears were evaluated according to morphological criteria for HPV: amphophilia, perinuclear halo, dyskeratosis, anisocytosis, nuclear criteria (binucleation or multinucleation), increased nucleus/cytoplasm ratio, anisokaryosis, hyperchromasia, nuclear atypia and karyorrhexis. In addition, the universally known Pap smear was performed. The detection of CIN was confirmed by colposcopy and biopsy in patients with cervical smears showing ASCUS, HSIL and LSIL [33].

Statistical analysis and ethical approved

Sample size was calculated as at least 300 (X^2 test with difference of 10%, power test of 80%). Chi-square and Fisher tests were used for statistical analysis with the significance level set at less than 0.05. This research was approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro.

Results

The percentage of infection in the first and second phases of the menstrual cycle was, respectively: Doderlein bacillus 52.49%/47.51%; cytolytic flora 61.59%/38.41%; *clue cell* 52.01%/47.98%; *Trichomonas vaginalis* 56.11%/43.89%; *Candida albicans* 47.60%/53.40%; coccoid bacillus 53.82%/46.18%; HPV 50.18%/49.82%; CIN I 52.15%/47.84%; CIN II 54.55%/45.45%; CIN III 47.37%/52.63%. Positivity of cytolysis and candidiasis were higher in the second phase of the menstrual cycle, with $p < 0.0001$ (Table 1). There was a superiority of the infection rate in the second phase of the menstrual cycle ($p < 0.05$) per cytolytic flora in women over 21 years old, *Candida albicans* between 21 and 50 years old and *Trichomonas vaginalis* between 41 and 50 years old (Tables 2-4).

When the presence of CIN associated with vulvovaginitis was evaluated, there was no significant difference in cytologies with CIN between the first and the second phases of the menstrual cycle (Table 5).

Discussion

The balance of the vaginal environment ecosystem is kept by the production of lactic acid. Lactobacilli inhibit the growing of other bacteria by different mechanisms. Among them the production of lactic acid, hydrogen peroxide and bacteriocin [35]. Lactobacilli occur in great numbers in the late luteal phase, prefer an acid environment and are frequently found in women using hormone therapy [33]. This is accord with our findings, in which we found a higher incidence of cytolytic flora in the second phase of the menstrual cycle. Exacerbation of the cytolytic flora can lead to cytolytic vaginosis, which symptoms are more pronounced during the lutheal phase of the menstrual cycle [36].

Vaginal pH during the proliferative phase of the menstrual cycle is higher than during the secretory one. Vaginal pH reaches very high values during menstruation [11, 37]. Low levels of estradiol and a high level of follicle-stimulating hormone are associated to higher values of vaginal pH [38]. In addition to hormone levels, other

Table 1. — *Absolute values (n) and percentages (%), correlating vaginal infections with phase of the menstrual cycle (proliferative and secretory phases.*

	Lactobacillus		Cytolytic flora*		Clue cells*		*Trichomonas vaginalis*		*Candida albicans**		Coccoid bacillus		HPV+	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Proliferative phase n = 7,730	5512	52.49	469	61.59	1378	52.01	271	56.11	1309	47.60	902	53.82	281	50.18
Secretory phase n = 7,130	4991	47.51	752	38.41	1271	47.98	212	43.89	1441	53.40	774	46.18	279	49.82
Total n = 14,860	1053	100	1221	100	2649	100	483	100	2750	100	1676	100	560	100

* $p < 0.0001$; +: Human Papillomavirus.

Table 2. — *Absolute values (n) and percentages (%) of positivity for cytolytic flora, according to phase of the menstrual cycle (proliferative and secretory phases).*

Age	Proliferative phase n = 7,730		Secretory phase n = 7,130	
	n	%	n	%
≤ 20	46	6.64	55	8.53
21-30*	117	5.92	188	10.04
31-40*	186	6.36	323	12.01
41-50*	113	5.65	172	9.42
≥ 51*	4	3.00	12	10.34

*$p < 0.05$.

Table 3. — *Absolute values (n) and percentages (%) of positivity for* Candida albicans, *according to phase of the menstrual cycle (proliferative and secretory phases).*

Age	Proliferative phase n = 7,730		Secretory phase n = 7,130	
	n	%	n	%
≤ 20	137	19.77	126	19.53
21-30*	376	19.02	443	23.66
31-40*	523	17.89	556	20.68
41-50*	258	12.90	300	16.43
≥ 51	11	8.27	12	10.34

*$p < 0.05$.

Table 4. — *Absolute values (n) and percentages (%) of positivity for* Trichomonas vaginalis, *according to phase of the menstrual cycle (first and second).*

Age	Proliferative phase n = 7,730		Secretory phase n = 7,130	
	n	%	n	%
≤ 20	24	3.46	13	2.01
21-30	58	2.93	46	2.46
31-40	106	3.62	99	3.68
41-50*	80	4.00	49	2.68
≥ 51	2	1.50	5	4.31

*$p < 0.05$.

Table 5. — *Absolute values (n) and percentages (%) of positivity for* clue cells, *associated with CIN, according to phase of the menstrual cycle (first and second).*

	CIN I *Clue-cells* positive		CIN I *Clue-cells* negative		CIN II *Clue-cells* positive		CIN II *Clue-cells* negative		CIN III *Clue-cells* positive		CIN III *Clue-cells* negative	
	n	%	n	%	n	%	n	%	n	%	n	%
Proliferative phase	29	56.86	80	50.96	7	41.18	23	60.53	9	36	56	52.34
Secretory phase	22	43.14	77	49.04	10	58.82	15	39.47	16	64	51	47.66
Total	51	100	157	100	17	100	38	100	25	100	107	100

factors can be related to the rise of vaginal pH in post-menopause, as the change of vaginal flora [39].

Variations in the levels of estrogen and progesterone in the menstrual cycle can influence changes in the immune response to Candida [30]. Vaginal candidiasis frequently occurs during pregnancy and during the luteal phase of the menstrual cycle, when there are high levels of estrogen and progesterone [40]. Our findings are in accordance with this in that a higher percentage of women had a diagnosis of vaginal candidiasis in the luteal phase of the menstrual cycle. Moreover, according to age group, there was a statistically significant difference for infection by cytolytic flora in women between 21 and 55 years old, and there was a statistically significant difference for infection by *Candida albicans* between 21 and 50 years old, which can be associated to the use of hormone contraception and hormone replacement therapy.

Cocobacillus increase in frequency after menopause, with the increase of the vaginal pH related to the increase of age. Therefore, an increase in cocobacillus in the first phase of the menstrual cycle is desirable when the pH is higher [35]. Our work shows a higher incidence in the first phase of the menstrual cycle, but this difference was not statistically significant.

Bacterial vaginosis is associated with sexual activity, sexually transmitted diseases [18] and smoking [41]. The vaginal environment ecosystem is altered in women with BV, with a reduction in the number of lactobacilli that produce hydrogen peroxide and a great increase in the number of facultative anerobic bacteria [42]. BV is the most common vaginal infection in young women, which is related to changing pH and the presence of clue cells in Papanicolaou smears [18]. Morison *et al.* [43] found a higher frequency of bacterial vaginosis in the first week of the menstrual cycle. Although the analysis was not carried out, there was the distinction of causative agents of bacterial vaginosis. In our study, we found a higher number of women with a diagnosis of *Gardnerella vaginallis* in the first phase of the cycle, but the difference was not statistically significant.

According to the age group, there was a statistically significant difference for infection by *Trichomonas vaginalis* between 41 and 50 years of age.

This work did not show a significant relation between the phase of the cycle and the presence of cytological alterations suggestive of HPV. Harper *et al.* showed that the phase of the menstrual cycle does not affect the rate

of HPV detection [44]. Garcia-Closas *et al.* demonstrated that elevated pH do not appear to be associated with risk of high-grade intraepithelial neoplasia in women infected with HPV [11].

Our results did not show any significant difference of CIN associated to clue cells in the first and second phases of the cycle. One would expect to find more frequent cytological findings of CIN and HPV in the first phase of the cycle with BV, since there is a relation between the less acid ph of the first phase and clue cells, HPV and CIN. Studies of the association of BV and CIN have had conflicting results. Boyle *et al.* showed that when HPV was present in association with BV, the incidence of CIN was much higher than with HPV of low risk which also had positive BV [25]. Guijon *et al.* reported that abnormal vaginal flora was significantly associated to CIN. Women with CIN have high concentrations of vaginal anerobics, more than those in the control group. The role of the development of CIN can not be directly related to microbial pathogenesis, but to products of the anerobic metabolism [45].

On the other hand, Peters *et al.* showed that in women with dyscariotic cervical smears, prevalence of BV did not increase, and BV did not influence any histological changes, probably not having any relation with the etiology of the cervical cancer [46]. Frega *et al.* showed that there was no significant correlation between CIN and BV; in contrast, there was a correlation with BV in patients without CIN. In the first group of patients affected by CIN and BV, BV was present in 36% of the cases, while in the second group of patients not affected by CIN, BV was present in 49% of the cases ($p < 0.00005$) [47].

Therefore, the frequency of cytolytic flora and *Candida albicans* is influenced by the phase of the menstrual cycle, but CIN is not.

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