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The length of menstrual bleeding and the risk of urogenital infections in the context of the activity of hemoglobin-derived microbicidal peptides

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Abstract

Objective: Well-known natural unspecific antimicrobial factors acting in the vagina are additionally reinforced during menstrual bleeding by hemocidins—a recently discovered novel class of microbicidal peptides generated proteolytically from hemoglobin. The aim of the presented research was to investigate the relation between the average length of menstrual bleeding and the frequency of urogenital infections. We expected that the shorter menstrual bleeding might increase the risk of urogenital infections because is synonymous with the shorter period of exposition on bactericidal action of hemocidins.
Study design: The study contains statistical analysis of an average declared length of menstrual bleeding in the group of 267 young, sexually active women with the symptoms of urogenital infections. The control group consisted of 300 young healthy women.
Results: The length of menstrual bleeding in the group of patients with urogenital infections (average 4.35 days) was statistically significantly shorter than in the control group (average 4.95 days). The average length of the menstrual cycle was equal and counted ca. 28 days in both groups.
Conclusion: The length of menstrual bleeding seems to be a significant factor in the vaginal ecology maintenance.

Keywords: Hemoglobin; Hemocidins; Antibacterial/antimicrobial peptides; Urogenital infections; Innate immunity; Reproductive immunology

1. Introduction

The well-balanced vaginal ecosystem is an important host defense mechanism against exogenous urogenital infections. Principal effectors of vaginal unspecific immunity include physical and physicochemical barriers (the epithelium, mucus, low pH, hydrogen peroxide), comensal microorganisms (predominantly *Lactobacillus* spp.), antibodies, cellular effectors as well as bactericidal proteins and peptides (lysozyme, lactoferrin, secretory proteinase inhibitor, elafin, cathelicidins and defensins) [1,2]. All the above factors provide a protective barrier from a variety of sexually transmitted pathogens that are more efficiently transmitted from man to woman than from woman to man.

Perturbations in the vaginal homeostasis increase the risk of bacterial infections, resulting in clinical syndromes, such as bacterial vaginosis (BV), the urinary tract infections (UTI) and others urogenital infections. For example, *G. vaginalis* is the predominant organism in the vaginal fluid from most women with nonspecific vaginitis. This anaerobic bacteria grows poorly in vitro in acidic pH [3] and pathological increase of pH causes an unusual growth of both this microorganism and anaerobic bacteria (including *Bacteroides* spp., *Peptococcus* spp., *Eubacterium* spp.). Similar consequences may also be observed in case of young sexually active women because it is well documented that the frequent use of spermicide as well as sexual intercourses are principal risk factors in the recurrent UTI's caused by *E. coli* [4,5]. *E. coli* is also a common opportunistic pathogen of the urogenital tract in the postmenopausal women. Recent observations prove that hormone replacement therapy causes restoration of the lactobacilli-dominant

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vaginal flora associated with its normal protective role against urogenital infections [6,7]. Generally, all these facts clearly demonstrate susceptibility of the vaginal ecosystem as well as its dynamic character. Urogenital infections which grow in number and become increasingly resistant to commonly used antimicrobial agents constitute an unresolved social problem.

According to our latest research mechanisms responsible for homeostasis in the female urogenital tract are additionally reinforced during menstruation by the so-called hemocidins—a novel class of microbicidal peptides generated proteolytically from hemoglobin [8]. This observation is an effect of detailed examination of the low-molecular weight proteins from the menstrual discharge of three healthy nulliparous women. The experiment showed that the analyzed material was exceptionally rich in hemoglobin fragments having bactericidal properties, particularly active against *E. coli*. Because a shorter bleeding period indicates a reduced time of the vagina's exposure to bactericidal action of hemocidins, it is interesting to elucidate the relationship between the length of menstrual bleeding and the frequency of infections of the genital and urinary tract. One might expect that shorter menstrual bleeding can increase the risk of urogenital infections because it is synonymous with a shorter period of exposure to bactericidal action of hemocidins. The aim of the presented cross-sectional epidemiological study was to investigate the relationship between the average length of menstrual bleeding and the frequency of urogenital infections. We hope that conclusions from our work will help in more adequate treatment of gynecological pathologies.

2. Materials and methods

2.1. Patients' characteristics

The study involved 567 female patients from a regional clinic in the Malopolska province (Poland, Europe), covering a population of 10 000 inhabitants. The study was carried out in the period between 2000 and 2005. Evaluation of patients was performed with comprehensive enrollment interviews, taking into consideration medical, obstetrical, gynecological, and sexual history. The study was approved by the Bioethical Committee of the Jagiellonian University in Kraków, agreement no. KBET/379/13/2003. In all cases we received patients' consent in writing.

Patients were divided into two groups. The first group contained 267 sexually active young women with urogenital infections, fulfilling the following particular criterion: presence of current symptoms of urogenital infections observed in the physical gynecological examination and in the laboratory tests (cytological examination, bacterial vaginosis test, pH of the vagina, bacterial culture of vaginal discharge and urine). This group did not contain women who: had no intercourse in the examined and previous cycle, used antibiotics, suffered from recurrent urogenital infections or concomitant infections. We also excluded women who used one of the following contraceptives: spermicides, oral contraceptives, cervical cups, intrauterine devices, condoms with or without spermicide. The length of menstrual bleeding as well as the length of the menstrual cycle was noted from interview.

The second group was a control group which comprised 300 healthy women consulted because of amenorrhoea in whom early pregnancy (4–6 week of pregnancy) was diagnosed. These had the same sexual habits as the women in the first group. Additionally, they did not use antibiotics because they did not suffer from urogenital infections. Similarly as in the above-discussed case, we recorded the lengths of menstrual stages from the interview and took into consideration the last menstrual period and the total length of the menstrual cycle before pregnancy.

2.2. Statistical analysis

Statistical calculations were performed using Statistica computer program (StatSoft, Poland). Data series were examined using Shapiro–Wilk and Student's *t*-tests, assuming normal sample distribution.

3. Results

Average values of general clinical parameters (such as age, body-mass index as well as average length of the menstrual cycle) in the healthy control group and in the infected group are presented in Table 1. The distribution of

Table 1
Comparison of clinical parameters among two groups of patients

	The healthy control	The patients with urogenital infections
No. of patients	300	267
Patients age (year ± S.D.)	26.2 ± 5.4 (range: 18-39)	26.3 ± 5.6 (range: 18–38)
Body mass index (kg/m^2 ± S.D.)	22.5 ± 2.35 (range: 18–26)	23.85 ± 3.26 (range: 18–29.7)
Parity (%)		
Nulliparity	50.7	74.9
Multiparity	49.3	25.1
Average length of menstrual cycle (day ± S.D.)	28.7 ± 4.09 (range: 21–54)	28.4 ± 2.03 (range: 21–35)