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Angaben zur Veröffentlichung / Publication details:

Kolben, T., K. Pieper, C. Goess, T. Degenhardt, Nina Ditsch, T. Weissenbacher, E. R. Weissenbacher, and T. M. Kolben. 2017. "IL-23, IFN-α, and IFN-β in the vaginal fluid of patients suffering from vulvovaginal candidosis." *Clinical and Experimental Obstetrics & Gynecology* 44 (1): 7–10. https://doi.org/10.12891/ceog3391.2017.

CEOG Clinical and Experimental Obstetrics & Gynecology

Original Articles

IL-23, IFN-α, and IFN-β in the vaginal fluid of patients suffering from vulvovaginal candidosis

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Summary

Purpose of the investigation: Vulvovaginal candidosis (VVC) is a common vaginal infection affecting almost 75% of all women once per lifetime. Vaginal associated immunity is important in the protection against VVC. The purpose of this study was to evaluate a potential role of IL-23, IFN-α, and IFN-β in the local immune response against VVC. *Materials and Methods:* The study included 202 non-pregnant women; 71 patients with clinical symptoms of VVC and 131 asymptomatic patients served as control. IL-23, IFN-α, and IFN-β were measured in the vaginal fluid by ELISA. Microbiological cultures were used for Candida detection. *Results:* C. albicans was detected in 67.6% of patients, C. glabrata in 21.1% of patients, and 5.6% were infected with C. krusei or coinfected with C. albicans and C. krusei. Levels of IL-23 ($p < 0.001$) and IFN-β ($p < 0.017$) were significantly lower in the VVC group. IFN-α was elevated in the VVC group compared to the asymptomatic patients ($p < 0.001$). *Conclusion:* IL-23 and IFN-β seem to play a protective role against VVC. Decreased levels in VVC patients suggest a compromised local immune response at the time of occurrence of symptoms. In contrast, IFN-α seems to be released once the infection has occurred. These cytokines may be prospective targets in the treatment and prevention of primary and recurrent vaginal infections with Candida species.

Key words: IL-23; IFN-alpha; IFN-beta; Vaginal fluid; Vulvovaginal candidosis.

Introduction

Vulvovaginal candidosis (VVC) is one of the most common infections of the female genital tract. Almost 75% of all women suffer from VVC at least once per lifetime [1-3]. In around 10% of these cases, women are affected by recurrent episodes of vaginal candida infection [4-6]. Most often the infection is caused by Candida (C.) albicans in about 85% of cases, followed by C. glabrata (4-5%), and other subspecies [1, 4].

Several factors predisposing for acute VVC are identified. Well known are immunosuppressive diseases, endocrine diseases (most importantly diabetes) and antibiotic therapy which causes an imbalance of the vaginal microflora [1, 2, 5-7]. Furthermore, there is evidence that an allergic predisposition and association with atopy might represent additional causative factors [8-10].

The vaginal defence mechanisms seem to act largely independently from the systemic immune system [11] which is supported by the observation that women suffering from recurrent VVC appear not to have higher susceptibility for candidosis of other mucous membranes as in the mouth or esophagus [12].

The cell-mediated immunity plays an important role in the local vaginal defence mechanisms against candida infections. T-lymphocytes represent major components of this immune response. They are activated by the presentation of antigens and different interaction between immune cells. This process is modulated by the release of cytokines.

Aside of the well known subtypes Th-1 and Th-2 T-helper cells, a third subtype, the Th-17-helper cells (Th17) was identified a few years ago. It is characterized by the production of IL-17 [13]. An increased production of Th17 was observed to be associated with several chronic inflammatory and autoimmune diseases [14, 15]. However, Th17 also seem to have protective capacities. They appear to provide protection against pathogens which are not sufficiently covered by Th1 or Th2-cells [16]. C. albicans was described to be one of these pathogens which mainly induced a Th17 response [17, 18]. In humans, IL-23, among others, induces Th17 differentiation from naive T cells [19]. The Th17 mediated immune response seems to be important especially in mucous membranes and epithelia [20].

Revised manuscript accepted for publication November 9, 2015

Table 1. — *Distribution of Candida subtypes in VVC patients.*

Candida subtype	Distribution n (%)
Total	71 (100)
C. albicans	48 (67.6)
C. glabrata	15 (21.1)
C. krusei	4 (5.6)
C. albicans + C. krusei	4 (5.6)

C.: Candida.

IFN-α and IFN-β are substantial components of the defence mechanisms against viruses [21]. Their role in the defence against other pathogens is explored to a lesser extent. However, there is evidence that IFN-α is involved in the defence against C. albicans [22].

Since antimycotic treatment does not always result in sufficient relief of VVC symptoms and the infection often reappears, there is reason to believe that underlying defects of the local immune system may be responsible. The purpose of this study was to evaluate whether an immunosuppression is already detectable in patients with symptomatic non- chronic VVC. Therefore, a potential role of the cytokines IL-23, IFN-α, and IFN-β was evaluated.

Materials and Methods

A total of 202 non pregnant patients were included in the present analysis. The test group consisted of 71 patients suffering from microbiologically confirmed VVC. 131 patients presenting for their gynecologic routine check up asymptomatic of VVC symptoms were included in the control group. All patients were interviewed regarding contraception, antibiotic use, history of STD's, and vaginal infections.

Exclusion criteria were as follows: pregnancy, use of antibiotics or antimycotics within the past 30 days, autoimmune or endocrine disorders, and no informed consent. Vaginal fluid was obtained and tested for the presence of candida species and the cytokines IL-23, IFN-α, and IFN-β.

Candida detection by culture

Vaginal secretion was inoculated on sabouraud agar and incubated for 48 hours at 37°C. Specification for candida species was done by inoculation of CHROM agar . CHROM agar allows selective isolation of yeasts and simultaneous identification of colonies of C. albicans, C. glabrata, C. krusei, and C. tropicalis. A distinctive colour of the colony after incubation for 24 hours at 37°C allowed the differentiation of each subtype [23].

Measurement of cytokines

A vaginal lavage was obtained by injecting two ml of physiological saline solution (NaCl) into the vagina and recovering two ml of the vaginal fluid, which was centrifuged. The supernatant was frozen at -80°C. After all samples were obtained, they were analyzed for the cytokines IL-23, IFN-α, and IFN-β.

The used ELISAs are solid phase sandwich enzyme linked-immuno-sorbent assays. A monoclonal antibody specific for IL-23 was coated onto the wells of the provided microtiter plates. Standards and samples were added to the appropriate wells. A standard curve was prepared with each assay.

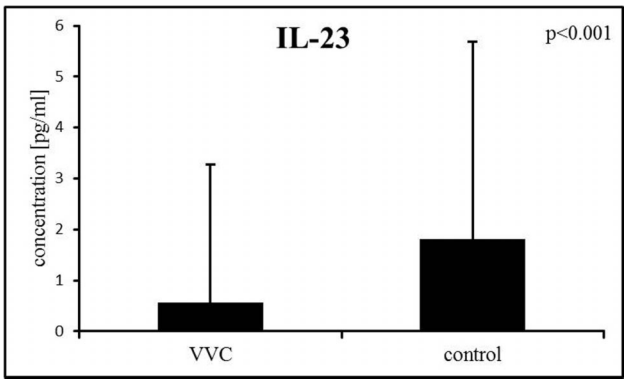

Figure 1. — Concentration of IL-23 in the vaginal fluid with vulvovaginal candidosis (VVC) (n=71) compared to healthy controls (n=100).

In case of IL-23, an anti-IL-23 biotinylated monoclonal antibody was added to all wells, except to those reserved for the chromogen blank. During incubation the antibody bonded to the immobilized hIL-23. After removing excess second antibody with wash buffer, streptavidin–HRP was added to each well. The wells were incubated again and after removing the entire unbound enzymes, a substrate solution was added to produce the colour reaction.

In case of IFN-α and IFN-β the protocol was similar, except that a HRP-conjugate specific for IFN-α or IFN-β was coated onto the wells of the provided microtiter stripes. After incubation substrate solution was added to produce the colour reaction.

The absorbance of the standards was plotted on a graph paper against the standard concentration. The intensity of produced colour was directly proportional to the cytokine-concentration.

Statistics

All analyses were carried out with the SPSS software. All tests were two sided using a significance level of 0.05.

Results

The distribution of Candida-subtypes in the VVC group was as follows: 48 patients (67.6%) tested positive for C. albicans, in 15 (21.1%) patients C. glabrata was detected, four patients (5.6%) were positive for C. krusei, and a coinfection with C. albicans and C. krusei was found in another four patients (5.6%) (Table 1).

Women suffering from symptomatic VVC were found to have significantly lower concentrations of IL-23 in their vaginal fluid than asymptomatic women of the control group ($p < 0.001$) (Figure 1).

The concentration of IFN-α was significantly increased in the vaginal fluid of patients with VVC compared to the control group ($p < 0.001$) (Figure 2). IFN-β was significantly decreased in the vaginal fluid of patients of the VVC group ($p = 0.017$) (Figure 3). No significant association between the concentrations of the cytokines in the vaginal fluid and the Candida subtype was detected by using the Kruskal-Wallis-test.

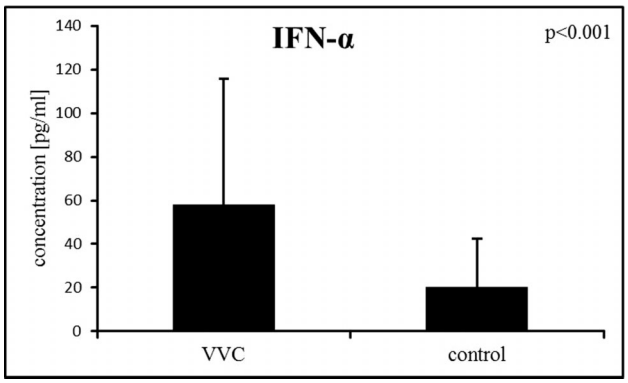

Figure 2. — Concentration of IFN-α in the vaginal fluid of patients with vulvovaginal candidosis (VVC) (n=71) compared to healthy controls (n=127).

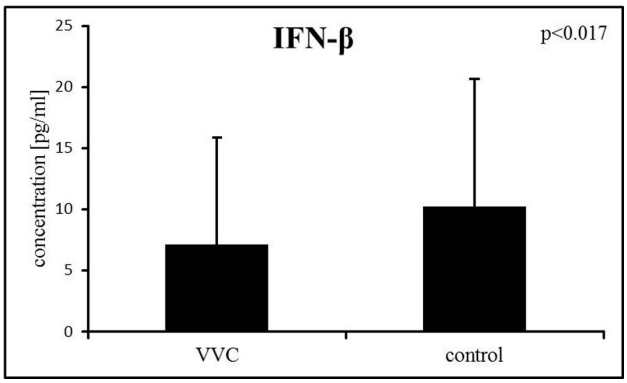

Figure 3. — Concentration of IFN-β in the vaginal fluid of patients with vulvovaginal candidosis (VVC) (n=71) compared to healthy controls (n=92).

Discussion

VVC represents a very frequent diagnosis of women affecting all age groups. More than 75% of all women suffer from at least one episode of VVC per lifetime [2, 5]. Around 10% of these women suffer from recurrent episodes [4, 5, 24]. VVC presents with pruritus, discharge, and burning and itching during urination and sexual intercourse [25]. These unpleasant symptoms do not only represent stress for the patients themselves, but also can be a burden for the patients' relationships.

In general, the transition from asymptomatic colonization to symptomatic VVC is supposed to be associated with a disruption or loss of the local defence mechanisms [26]. Anton *et al.* observed that cell-mediated immunity plays an important role in vaginal bacterial infections [27]. Elevated concentrations of the anti-inflammatory cytokine IL-4 in the vaginal fluid of recurrent VVC patients were detected, which indicates a localized vaginal immunosuppression [28]. This finding is in accordance with the results of this analysis. The hypothesis of a compromised local immune system is confirmed by the finding of decreased IL-23 levels in the vaginal fluid of symptomatic VVC patients. IL-23 is produced by antigen presenting cells as an unspecific reaction to an infection with fungi [29, 30]. Especially, C. albicans seems to induce the release of IL-23 [31] and thereby the differentiation and proliferation of Th17 [19]. Acosta-Rodriguez *et al.* observed that C. albicans specific T-helper memory cells were Th17-lymphocytes [31]. The Th17 mediated immune response was described to be important especially on mucous membranes and epithelia [20, 32]. Wu *et al.* were able to detect higher levels of IL-23 mRNA in the vagina of immune-competent mice infected with C. albicans compared to immune-suppressed mice which supports the findings in the current study [33].

Limitation of the present analysis is that a history of prior VVC in the asymptomatic patients was not assessed. Since these patients all tested negative for Candida, it remains unclear whether they have higher IL-23 levels due to prior contact to Candida. It is also possible that their IL-23 levels are elevated per se which prevents an infection with Candida in the first place.

The antiviral function of IFN-α and IFN-β is largely studied. Type I-IFNs have immune-modulating functions. On the one hand, they display anti-inflammatory effects as therapeutic agents against a specific form of multiple sclerosis. On the other hand, however, proinflammatory side effects of an IFN therapy have also been described. The knowledge about the role of those Type I-IFN in the defence against Candida is less extensive. However, there is evidence that both IFN are involved in this process [22, 34, 35]. After iv-infusion of Candida, mice lacking the IFN-α/β receptor died from their inability to control fungal growth, whereas all wild type controls survived. These data suggest an important role of a IFN-α/β response in the protection against candida [34].

In this analysis the levels of IFN-α in patients with VVC were significantly increased. IFN-α can suppress the expression of IL-17 as well as the differentiation of naive T-helper cells to Th17 [36]. In a mouse model, reduced expression of IL-17 in the vaginal fluid was associated with an exacerbation of the Candida infection [37]. Thereby, the essential role of Th17 in the defence against Candida would be inhibited.

Smeekens *et al.* reasoned from their observations in a cell culture model that Type I-IFNs most likely IFN-β modulate the immune reaction induced by C. albicans, directing the defence towards a Th1 response [22]. This is accordance with the findings of another group who discovered the important role of IFN-β signaling in modulating the host immune response [35]. The in vivo observation of decreased IFN-β levels in symptomatic VVC patients confirms these suggestions.

Conclusion

The cytokines IL-23 and IFN-β could be shown to be significantly decreased in the vaginal fluid of VVC patients, suggesting a protective role against VVC. In contrast, IFN-α which can inhibit Th17, a major factor in the defence against Candida, was significantly increased.

To the present authors' knowledge, this is the first analysis in the current literature which could show significant changes of these cytokines in the vaginal fluid of VVC patients. These in vivo data support the hypothesis of compromised local defence mechanisms against an infection with Candida in patients suffering from VVC. Supporting the local immune system may be a benefical future therapeutic approach against VVC.

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