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Landscape of *in situ* cytokine expression, soluble C-type lectin receptors, and vitamin D in patients with recurrent vulvovaginal candidiasis

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Abstract

The immunopathogenesis of recurrent vulvovaginal candidiasis (RVVC) is poorly understood. Recently, it was reported that patients with RVVC present a decrease in both the fungicidal capacity of neutrophils and the proliferative capability of peripheral blood mononuclear cells in response to *Candida albicans* infection, suggesting an alteration in the innate and adaptive immune response. The aim of this study was to determine the in-situ expression, in the vaginal mucosa, of genes associated with the immune response, as well as the serum concentrations of dectin-1, mannose-binding lectin (MBL), and vitamin D in patients with RVVC. A study was carried out on 40 patients with a diagnosis of RVVC and 26 healthy women. Vaginal scrapings were obtained, and the expression of genes that encode cytokines and transcription factors specific for Th1, Th2, Th17, Treg, pro-inflammatory profiles, and enzymes related to oxidative/microbicidal mechanisms was evaluated by quantitative polymerase chain reaction (qPCR). Additionally, serum levels of vitamin D and the soluble receptors dectin-1 and MBL were determined by enzyme-linked immunosorbent assay (ELISA). In patients with RVVC, a decreased expression of T-bet, RORγ-T, IL-1β, and IL-17, and an increase in the expression of FOXP3, IL-4, IL-8, IL-10, and IL-18 were observed when compared to healthy women: moreover, decreased levels of MBL were also observed in these patients. These results confirm that patients with RVVC present in-situ alterations in both the specific and adaptive immune response against *Candida* spp., a fact that could be associated with the exaggerated vaginal inflammatory response.

Lay summary

The study concerns the immune response of women with recurrent vulvovaginal candidiasis; we observed an alteration in the expression of genes that participate in the control of infection, a fact that could be associated with the exaggerated vaginal inflammatory response observed in those patients.

Key words: RVVC, immune response, cytokines, MBL, vitamin D.

Introduction

Vulvovaginal candidiasis (VVC) is the second most common cause of infections in the female genito-urinary tract, affecting approximately 75% of the world's population at least once in their life.[1] Along these lines, VVC affects not only women on a physiological but also a psychological, sexual, and social level.[2–4] Additionally, there is a phenomenon known as recurrent vulvovaginal candidiasis (RVVC), which is defined as the presentation of at least four episodes of VVC in a year.[5] On the other hand, both VVC and RVVC are infections with multifactorial compounds, where underlying conditions such as uncontrolled diabetes mellitus, the luteal phase of the menstrual period, pregnancy, the use of broad-spectrum antibiotics, contraceptive therapy, the use of corticosteroids, and behaviors such as the use of vaginal douching, in addition to alterations in the immune response, are considered risk factors associated with the development of these conditions.[6,7] Regarding the latter, it is believed that the host's immune response could play a key role in the pathogenesis of RVVC; some authors even consider it an auto-inflammatory disease or an immunodeficiency condition.[6] In this sense, once *Candida* spp. begins the process of transition from a harmless or commensal state to a pathogenic one, in some species the formation of hyphae is promoted, in addition to the secretion of toxins such as candidalysin, considered an important virulence factor in yeasts of the genus *Candida*.[8] Additionally, cells such as neutrophils initiate the recognition of this fungus through pathogen-associated molecular pattern receptors (PRRs), mainly through TLR-2, TLR-4, dectin-1, and mannose-binding protein (MBL).[9] Regarding dectin-1, it has been reported that it has a protective role in fungal infections that include *Candida albicans*, *Aspergillus fumigatus*, and *Pneumocystis jirovecii*.[10]

Likewise, it has been observed that MBL also plays a pivotal role in protection against infections by *Candida* spp.; thus, reduced levels of MBL and a higher frequency of polymorphisms

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in the gene that codes for this protein in women with RVVC have been reported.[11–14]

Similarly, TCD4 + lymphocytes with Th1 and Th17 profiles, as well as the transcription factors and cytokines derived from these profiles, are associated with a protective effect against this type of infection,[6,15] while cells or cytokines associated with a Th2 or Treg profile are often associated with deleterious effects on the immune response during the development of infections by mycobacteria or pathogenic fungi, including *Candida* infections.[6,15]

On the other hand, some authors have reported that vitamin D seems to have an important role in the defense against infections by *Candida* spp. since it could induce the production of antimicrobial peptides such as cathelicidin, which has action against fungi, bacteria, and mycobacteria in various anatomical sites including the skin and mucous membranes.[16–18]

Recently, we demonstrated that patients with RVVC present a decrease in both the microbicidal capacity of neutrophils and the proliferative capability of peripheral blood mononuclear cells against *Ca. albicans*, suggesting a compromise of the innate and adaptive immune response in these patients.[19] Taking the above into account, the aim of this study was to characterize the immune response in patients with RVVC by determining the expression, *in situ* in the vaginal mucosa, of genes associated with different immunological profiles, as well as the serum levels of the vitamin D and pathogen-associated molecular pattern recognition receptors dectin-1 and MBL.

Methods

Ethical aspects

This study was approved by the Human Research Bioethics Committee (Acta No. 21-28-935), of the University Research Headquarters (SIU) of the Universidad de Antioquia.

Population and type of study

A descriptive cross-sectional study was carried out where 66 women between 18 and 50 years old were included, divided into two groups: 40 women with a confirmed diagnosis of RVVC and 26 healthy women (control group). In the group of women diagnosed with RVVC, 37 had an active infection with *Candida albicans* and three with *Ca. lusitaniae*. Women who were pregnant or who had been under treatment with antibiotics, antifungals, or corticosteroids in the last 7 days before the sample was taken were excluded from the study; in addition, women who were under hormone replacement therapy or who had any underlying disease such as diabetes, autoimmune diseases, Human Immunodeficiency Virus (HIV) infection, cancer or another condition were also excluded. Once the inclusion criteria of the study were met, the participants were given informed consent for subsequent signing and collection of clinical samples.

Sample collection and procedures

Scraping samples of the vaginal mucosa were obtained from all participating women, both those with a diagnosis of RVVC and healthy women; sampling was carried out through the use of sterile specula and cytobrushes. The samples were inoculated into tubes with sterile saline solution and centrifuged at 10 000 x *g* for 10 min; the supernatant was discarded and the sediment was resuspended in 200 µl of TRIZol (InvitrogenTM, ThermoFisher Scientific, USA); finally, the samples were frozen at -80°C for subsequent gene expression analysis.

Additionally, a blood sample was obtained using tubes without anticoagulants using previously standardized venipuncture procedures. Subsequently, the sample was incubated for 1 h at 37°C, followed by centrifugation for 10 min at 300 x *g*. Finally, serum was obtained and stored at -80°C for ELISA assays.

Ribonucleic acid (RNA) extraction

RNA extraction was performed on the vaginal scraping samples previously resuspended in TRIZol (InvitrogenTM, ThermoFisher Scientific, USA), using the commercial E.Z.N.A.® Total RNA Kit I (Omega Bio-Tek, USA) and following the manufacturer's instructions. Additionally, integrity, quantity, and quality criteria of the RNA were evaluated by agarose gel electrophoresis and spectrophotometry using the NanoDrop One equipment (ThermoFisher Scientific, USA) and considering the absorbance ratios 260/230 and 260/280.

Synthesis of complementary deoxyribonucleic acid (cDNA)

The previously extracted RNA samples were subjected to a DNase treatment process using DNase I Amplification Grade (1 unit/µl) (Sigma Aldrich, USA). Subsequently, cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, ThermoFisher Scientific, USA), following the manufacturer's instructions. The quantity and quality of the samples were evaluated using the NanoDrop One equipment (ThermoFisher Scientific, USA) as previously described. Finally, the cDNA samples were stored at -20°C for further analysis.

Gene expression analysis

Both the primers for β-actin and the genes of interest were designed de novo and synthesized by Merck (USA). The genes and primer sequences used in this study are listed in Table 1. qPCR reactions were carried out using the HOT FIREPol® EvaGreen® qPCR Supermix kit (Solis BioDyne, Estonia) and the CFX96 Touch Real-Time PCR (Bio-Rad, USA). For each reaction, a final volume of 15 µl was analyzed. The amplification protocol was carried out as follows: an initial cycle of polymerase activation for 12 min at 95°C, followed by 40 amplification cycles for 15 s at 95°C, in addition to an annealing and extension phase for 30 s each. Additionally, the relative expression of the genes of interest was calculated using the 2-ΔΔCt method, considering the Ct (cycle threshold) values normalized against β-Actin used as a constitutive gene.

Determination of serum concentrations of dectin-1, MBL, and vitamin D

Serum concentrations of the soluble recognition receptors dectin-1 and MBL (Invitrogen, USA), and vitamin D (Novus Biologicals, USA) were determined using specific commercial ELISA kits and following the manufacturers' instructions.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 9.0 (Dotmatics, USA). The Shapiro–Wilk test was used to evaluate the criterion of normality in the distri-

Table 1. List of genes and primer sequences used for qPCR analyses.

Gene	Primer	Nucleotide sequence (5´-3´)
IL-8	IL-8-F	TTTTGCCAAGGAGTGCTAAAGA
	IL8-R	AACCCTCTGCACCCAGTTTTC
IL 1β	IL 1β-F	CTACGAATCTCCGACCACCAC
	IL 1β-R	GGTCATTCTCCTGGAAGGTCTG
IL 18	IL 18-F	ACCTCTACAGTCAGAATCAGTCA
	IL 18-R	TGGAATCAAGATTACTTTGGCAAGC
TNF-α	TNF α-F	ACCTCATCTACTCCCAGGTCC
	TNF α-R	GCTCTTGATGGCAGAGAGGAG
TBET	TBET-F	TCCCATTCCTGTCATTTACTGTGG
	TBET-R	CCTTTCCACACTGCACCCACT
IFN-γ	IFN γ-F	GGTAACTGACTTGAATGTCC
	IFN γ-R	TTTTCGCTTCCCTGTTTTAG
RORγ-T	RORγ T-F	CAGGATGCCTGTGTTCCTCA
	RORγ T-R	GCCACCTTGAATACAGCCCT
IL 17A	IL 17A-F	CTACAACCGATCCACCTCACC
	IL 17A-R	AGCCCACGGACACCAGTATC
FOXP3	FOXP3-F	GTGGCATGGGGTTCAAGGAA
	FOXP3-R	GGAGGGCTGCACCCAAAG
TGF-β1	TGF β1-F	AACTATTGCTTCAGCTCCACGG
	TGF β1-R	GGCAGAAGTTGGCATGGTAG
IL 10	IL 10-F	CTCGAAGCATGTTAGGCAGGT
	IL 10-R	GCTCAGCACTGCTCTGTTGC
IL 4	IL 4-F	TCTTTGCTGCCTCCAAGAACAC
	IL 4-R	CTCTGGTTGGCTTCCTTCACAGG
GATA3	GATA3-F	TCTCAGCCCCTTCTCCAAGA
	GATA3-R	GGAAGGTGAAGAGGTGCGG
IDO1	IDO1-F	CTGGAAAGGCAACCCCC
	IDO1-R	AGGACGTCAAAGCACTGAAAG
iNOS	iNOS-F	TGCCCATGTACCAGCCATTG
	iNOS-R	ACGAGTGGTTTCGGGAACTG
NOX2	NOX2-F	GAGTTGTCATCACGCTGTGC
	NOX2-R	ATGGATGGCAAGGCCAATGA
β-ACTIN	β-ACTINA-F	TGTAGAAGGTGTGGTGCCAG
	β-ACTINA-R	GGGCATGGGTCAGAAGGATT

bution of the data. Those data that did not present a normal distribution were analyzed using the Mann–Whitney U test, and those that presented a normal distribution were analyzed using the *t* test for unpaired data. Finally, to describe the variables, the standard error of the mean (SEM) was considered; and *P*-values $< .05$ indicated statistically significant differences between the data.

Results

Patients with RVVC present a decrease in the expression of the Th1 and Th17 profiles and an increase in the expression of the Th2 profile

Gene expression analyses were performed *in situ* in the vaginal mucosa of women with a clinical and microbiological diagnosis of RVVC. In these patients, a significant decrease in the expression of the transcription factor T-bet, considered a specific transcription factor for the Th1 immunological profile, was observed compared to the control group ($P < .05$); however, the expression of the gene that codes for Interferon-gamma (IFNγ) did not show any difference between women with RVVC and the control group (Fig. 1A).

Regarding the Th17 profile, women with RVVC presented a significant decrease in the expression levels of the transcription factor RORγt and IL-17 compared to the control group ($P < .05$) (Fig. 1B).

On the other hand, in patients with RVVC a significant increase in the expression of the gene that codes for IL-4, considered a specific cytokine of the Th2 immunological profile, was observed when compared to the control group ($P < .05$). It is noteworthy that the expression of the gene encoding the GATA3 transcription factor (a specific transcription factor of the Th2 immune profile) did not show any difference between women with RVVC and the control group (Fig. 1C).

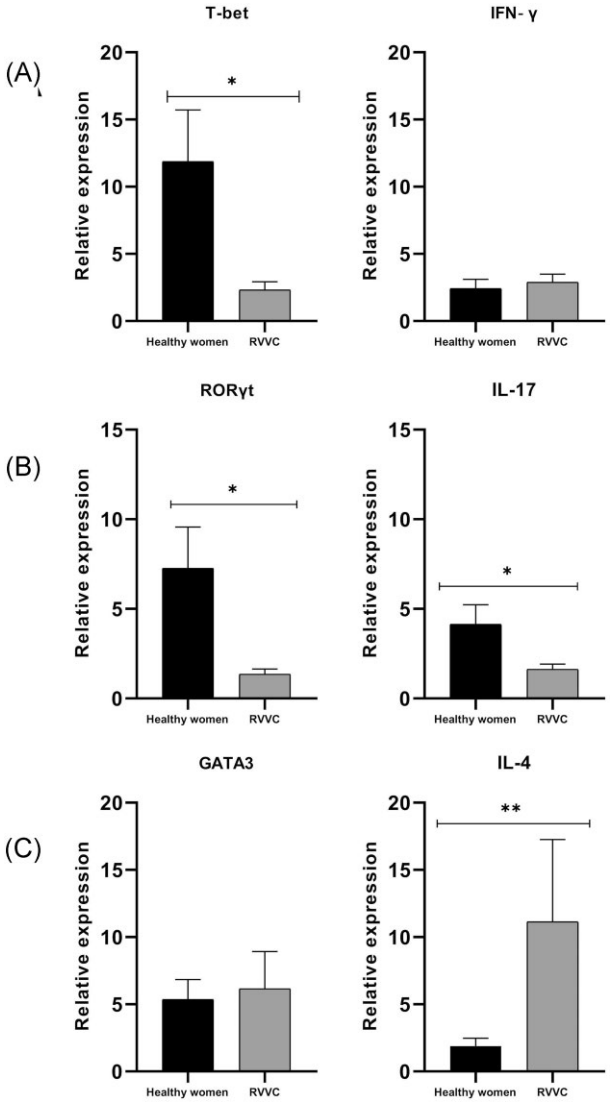

Figure 1. Expression of genes associated with immunological profiles in vaginal mucosa of patients with RVVC ($n = 40$) and healthy women (control group, $n = 26$). (A) Analysis of the expression of genes associated with the Th1 profile (T-bet and IFNγ). (B) Analysis of the expression of genes associated with the Th17 profile (RORγt and IL-17). (C) Analysis of the expression of genes associated with the Th2 profile (GATA3 and IL-4). Results are expressed as the mean ± standard error of the mean (SEM). $^{*}P < .05$ and $^{**}P < .01$.

Patients with RVVC present an alteration in the expression of genes encoding for pro-inflammatory cytokines

In this study, cytokines associated with a pro-inflammatory profile (IL 1β, IL 8, IL 18, and Tumor necrosis factor ralfa - TNFα), which play an important role in the *in situ* inflammatory response in the vaginal mucosa were evaluated. In patients with RVVC, a significant decrease in the expression of

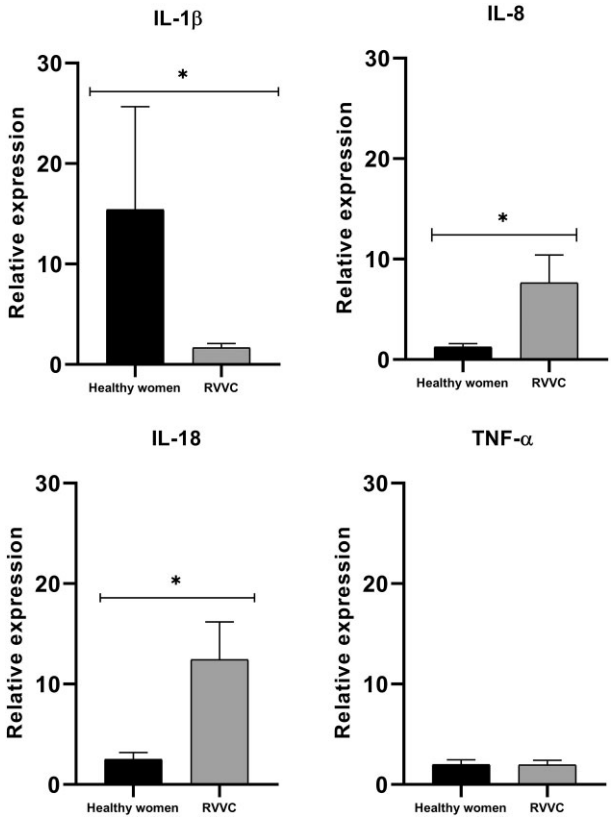

Figure 2. Expression of genes associated with an inflammatory profile in vaginal mucosa of patients with RVVC ($n = 40$) and healthy women (control group, $n = 26$). Analysis of the expression of genes associated with an inflammatory profile (IL-1β, IL-8, IL-18, and TNFα). Results are expressed as the mean ± standard error of the mean (SEM). *$P < .05$.

the gene that codes for IL-1β and a significant increase in the expression of the genes that code for IL-8 and IL-18 were observed compared to the group control ($P < .05$). However, no difference was observed in the expression of the gene encoding for TNFα between the groups evaluated (Fig. 2).

Patients with RVVC present an increase in the expression of genes associated with regulatory T cells

In relation to the evaluation of gene expression in transcription factors and cytokines associated with a profile of regulatory T cells (Treg), a significant increase in the expression levels of FOXP3 ($P < .05$) and IL-10 ($P < .01$) was observed in the group of patients with RVVC compared to the control group. For TGFβ-1, no significant difference in expression levels was observed between the groups evaluated (Fig. 3).

Patients with RVVC do not present alterations in the expression of genes associated with oxidative/microbicidal mechanisms

Additionally, the expression levels of genes associated with oxidative/microbicidal mechanisms were evaluated. The genes that encode the enzymes IDO1 (indolamine 2,3-dioxygenase 1, a cytosolic enzyme that degrades the amino acid L-tryptophan and has been associated with microbicidal mechanisms and regulators of the immune response), iNOS (inducible nitric oxide synthase, associated with the production of nitric oxide and reactive nitrogen species), and NOX2 (NADPH oxidase-2, associated with the production of reactive oxygen species) were evaluated; however, no differences were observed in these genes between women with RVVC and the control group (Fig. 4).

Patients with RVVC have decreased levels of MBL but not of dectin-1 or vitamin D

Considering that soluble pathogen-associated molecular pattern recognition receptors dectin-1 and MBL, in addition to vitamin D, play a central role in the recognition and defense against *Candida* spp., serum levels of these molecules were evaluated both in healthy women and in patients with RVVC. Of note, significantly decreased levels of MBL were observed in the RVVC patients compared to healthy women ($P < .001$). On the contrary, no significant differences were found between the serum concentrations of dectin-1 or vitamin D in the groups evaluated (Fig. 5).

Discussion

In a previous study conducted by our research group in women with a clinical and microbiological diagnosis of RVVC, it was reported that 92.5% (37 patients) had *Candida albicans* infection and 7.5% (three patients) had *Clavispora lusitaniae* (formerly *Ca. lusitaniae*) infection with less than 10% resistance to the most used antifungals.[19] Notably, it was also found that these patients presented a decrease in the microbicidal capacity of neutrophils and the proliferative capability of peripheral blood mononuclear cells against *Ca. albicans*, thus suggesting a compromise of the innate and adaptive immune response. Therefore, in the present study, immunological characterization was performed *in situ*, in the vaginal mucosa, in those patients with RVVC.

Regarding the adaptive immune response, it is well known that Th1 lymphocytes have a pivotal role in the protective response against *Candida* spp.; thus, in this study, the expression of T-bet, a specific transcription factor that induces and modulates the differentiation of naïve T cells to a Th1type immunological profile,[20] as well as the expression of IFNγ, a hallmark cytokine linked to this immunological profile were evaluated. Thereby, a significant decrease in this transcription factor was found, suggesting a possible alteration of the Th1 type response in patients with RVVC; however, no differences were found in the expression of IFNγ between patients with RVVC and the control group. These findings correlate with what was found in a study by Bai et al.[21] in which the authors evaluated, in a mouse model, a lethal systemic infection model by *Ca. albicans*, and found a decrease in the expression of T-bet, suggesting that this transcription factor plays an important role in the regulation of CD4 + T cells (Th1) during candidiasis.

On the other hand, RVVC symptomatology is associated with the chronic inflammatory state experienced by these patients.[6] In accordance with the above, in the RVVC group evaluated in this study, an increase in the expression of proinflammatory cytokines such as IL-8 and IL-18 was observed, these being important in the process of chemotaxis and recruitment of neutrophils in the vaginal mucosa, which translates into a positive feedback mechanism that results in the hyperinflammatory state characteristic of this infection.[22] In line with our findings, Lilic et al., reported that blood cells from

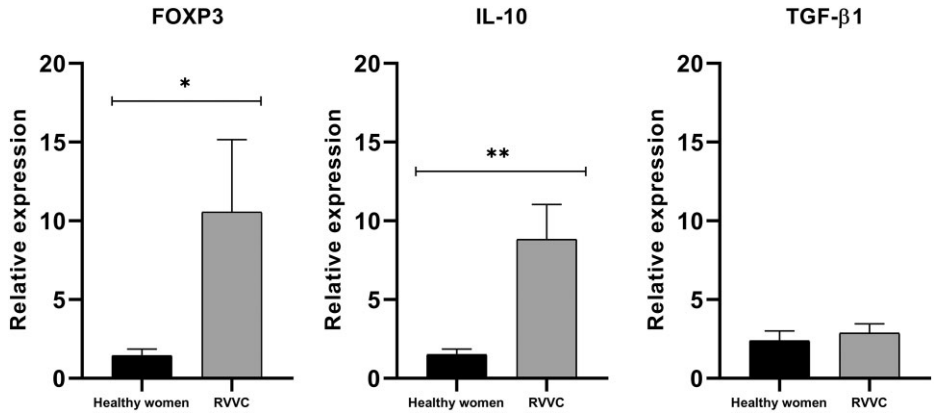

Figure 3. Expression of genes associated with a profile of regulatory T cells in vaginal mucosa of patients with RVVC ($n = 40$) and healthy women (control group, $n = 26$). Analysis of the expression of genes associated with a Treg cell profile (FoxP3, IL-10, and TGF-β1). Results are expressed as the mean ± standard error of the mean (SEM). $^{*}P < .05$ and $^{**}P < .01$.

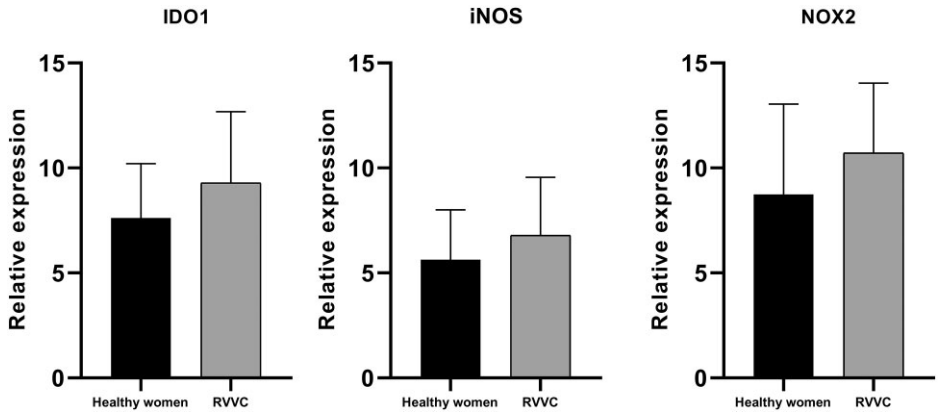

Figure 4. Expression of genes associated with oxidative/microbicidal mechanisms in vaginal mucosa of patients with RVVC ($n = 40$) and healthy women (control group, $n = 26$). Analysis of the expression of genes associated with oxidative/microbicidal mechanisms (IDO1, iNOS2, and NOX2). Results are expressed as the mean ± standard error of the mean (SEM).

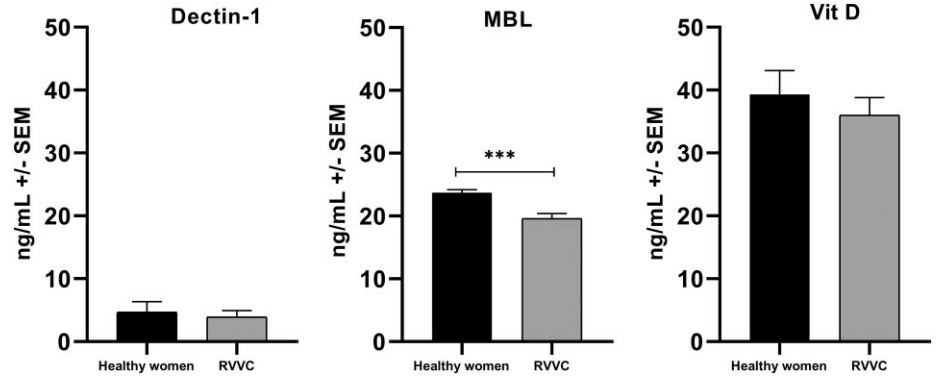

Figure 5. Serum levels of soluble pathogen-associated molecular pattern recognition receptors and vitamin D in patients with RVVC ($n = 40$) and healthy women (control group, $n = 26$). The levels of soluble dectin-1, MBL, and vitamin D were determined in the serum of patients with RVVC and healthy women by means of the ELISA technique. Results are expressed as the mean ± standard error of the mean (SEM). $^{***}P < .001$.

patients with chronic mucocutaneous candidiasis, when stimulated with different fractions of *Ca. albicans*, did not show changes in TNF-alpha production, and suggested that this effect could be related to a defect in macrophages or dendritic cells[23]; but contrary to what was reported by Rosati et al., who observed that monocytes from RVVC patients infected with *Ca. albicans* hyphae presented increased levels of TNF-α, in the present study no differences were observed in the expression of this cytokine in the vaginal mucosa.[24]

The adaptive immune response mediated by the Th17 immune profile, through the production of IL-17, seems to have a key role in the control of fungal infections in the skin and mucosa through the production of antimicrobial peptides and recruitment and activation of neutrophils; however, its role

in VVC and RVVC is controversial.[25,26] Recently, in a mouse model of RVVC, it was shown that IL-17 appears to play a protective role through the recruitment and activation of neutrophils.[27] In the present study, patients with RVVC showed decreased expression levels of IL-17 and the transcription factor RORγT associated with this Th17 profile, which is consistent with recent findings in which it has been confirmed that alterations in the Th17 type response can promote the development and recurrence of mucosal infections due to *Ca. albicans*.[28]

It has been observed that Treg cells play a fundamental role in controlling the immune response against some pathogens including *Candida* spp. In this sense, the expression of the FOXP3 transcription factor is associated with this profile; and it has been described that Treg cells may have a dual role during some fungal infections, for example, either as an immune evasion mechanism or promoting the elimination of potential pathogens.[29,30] Therefore, the role of Treg cells in candidiasis is difficult to predict; however, in a mouse model of disseminated candidiasis, it has been observed that populations of CD4+/CD25 + T lymphocytes (Treg) cells have the ability to inhibit the function of effector cells such as macrophages, through the production of IL-10, one of the main cytokines with anti-inflammatory effect, promoting the development of infection.[31] The above correlates with the results observed in the present study, where a significant increase in the expression of IL-10 and FOXP3 was evident in patients with RVVC.

Regarding the Th2 cellular profile, it is well known that it promotes the humoral immune response and exerts an antagonistic effect by suppressing cellular or Th1-type immunity. This profile is associated with the production of cytokines such as IL-4 and IL-5, which are considered by some authors to be key factors for the development of RVVC.[32–34] In line with the above, in this study, a significant increase in IL-4 expression was observed in the group of patients with RVVC; but contrary to the results obtained in the present study, Rosati et al. evaluated the response of monocytes from RVVC patients infected with *Ca. albicans* hyphae, and did not observe a difference in the production of cytokines associated with Th1, Th2, or Th17 profiles compared to infected monocytes from healthy women.[24] Thus, the above results suggest that the immune response appears to be compartmentalized and differs according to the tissue or cell studied. Moreover, it seems that innate and adaptive lymphocytes utilize distinct enhancer elements for their development and differentiation, and additionally, the regulation of a single cytokine is controlled by distinct transcriptional mechanisms within the T cell lineage.

In relation to microbicidal mechanisms, exerted mainly by phagocytic cells, oxidative mechanisms dependent on oxygen and nitrogen, among others, have been described. In this sense, the expression of three enzymes related to microbicidal/oxidative capacity was determined: IDO1, iNOS, and NOX2, NADPH oxidase-2. However, no significant differences were observed in the expression of these molecules between the two groups evaluated, which is interesting taking into account that other authors have reported that NOX2 is a critical protein for the elimination of fungal pathogens including *Ca. albicans*.[35] Furthermore, it has been described that IDO1 deficiencies in mice can promote an intrinsic susceptibility to infections by *Candida* spp. in mucous membranes.[36] On the other hand, in a study in which a model of oral candidiasis was used in NOS2-deficient mice (NOS2-null) and wild-type mice, no differences were observed in aspects such as the effectiveness in eliminating the fungus, the response associated with host cytokines, or the fungicidal activity exerted by macrophages against *Ca. albicans*.[37] The above allows us to suggest that in patients with RVVC, the enzymes IDO1, NOS2 (iNOS), and NOX2 seem not to have a critical role in the immune response against this infection. However, in a previous study by our group, it was reported that neutrophils from patients with RVVC exhibit a decrease in fungicidal capacity against *Ca. albicans*,[19] which suggests that microbicidal mechanisms other than those associated with the three enzymes studied here could be affected in these patients with RVVC.

On the other hand, in agreement with the findings obtained by Rosentul et al.,[38] in the present study no significant differences were found in the serum concentrations of dectin-1 between healthy women and patients with RVVC. This could be explained from a genetic point of view in which homozygous individuals with dectin-1 deficiency are considered rare in the Western population,[38] indicating that dectin-1 deficiency might not be responsible for the susceptibility to developing RVVC at the population level but rather at the individual level. In relation to the soluble MBL receptor, in a study carried out by Babula et al., decreased levels of this protein were found in patients with RVVC compared to healthy women,[13] which agrees with the findings of the present study. This is relevant, taking into account that MBL is a soluble receptor that circulates in peripheral blood and is an important component in the innate immune system, which recognizes and binds mannose and N-acetyl-glucosamine residues present on the surface of microorganisms that include *Candida* spp., resulting in complement activation.[14] Additionally, other studies have reported the presence of polymorphisms in the gene that codes for the MBL protein and decreased levels of the soluble protein, mainly in patients with RVVC.[39,40] Therefore, a decrease in serum MBL levels could be associated with alterations in the innate immune response that can lead to the development of recurrent infections,[41] as is the case in patients with RVVC.

Finally, vitamin D deficiency has been associated with *Candida* infections in the oral mucosa, especially in patients living with HIV. This molecule exerts immunoregulatory functions once it binds to its receptor present in different cell populations including B lymphocytes (LB), T lymphocytes (LT), monocytes, and macrophages.[42,43] In the case of RVVC, there are no studies on the role of this vitamin in the physiopathogenesis of this affection; however, a few studies have indicated a potential relationship between vitamin D3 deficiency and the risk of suffering VVC,[18] which differs from the results obtained in this study, in which no significant differences were found in vitamin D levels between women with RVVC and the control group.

In conclusion, in the present study, it was observed that women with RVVC, at the level of the vaginal mucosa, present a decrease in the *in situ* expression of cytokines and transcription factors associated with the Th1 and Th17 immunological profiles, which seem to have a protective effect in this pathology. Additionally, an increase in the expression of pro- and anti-inflammatory cytokines was evident, which could be associated with the exacerbated and ineffective chronic inflammatory response observed in these patients; furthermore, decreased levels of soluble MBL were also observed. Together, these findings provide evidence and confirm an alteration in

both the innate and adaptive immune responses in patients with RVVC.

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Author contributions

Jeiser Marcelo Consuegra-Asprilla (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft, Writing - review & editing), Manuela Chaverra-Osorio (Formal analysis, Investigation, Methodology, Writing - original draft), Brajhan Torres (Formal analysis, Investigation, Methodology, Writing - review & editing), Yuliana Cabrera-Chingal (Formal analysis, Investigation, Methodology, Writing - review & editing), Angelica Mancera-Mieles (Formal analysis, Investigation, Methodology, Writing - review & editing), Carolina Rodríguez-Echeverri (Data curation, Formal analysis, Investigation, Methodology, Writing - review & editing), Beatriz L. Gómez (Formal analysis, Funding acquisition, Investigation, Validation, Writing - review & editing), and Ángel González (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing).

Supplementary material

Supplementary material is available at *Medical Mycology* online.

Conflict of interest

The authors declare no conflict of interest.

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