



Expression of Cytokines in Vaginal Secretion of Women with Yeast Colonization

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LNOC and OKO designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors CN, ATOA and MAAW managed the collection of the samples and contributed in the analyses of the specimen. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Comparative analysis of local expression of IL-2, IL-10 and TNF- α was carried out to provide an insight to the extent and type of localized immune response associated with yeast colonization of the urogenital tract.

Study Design: Descriptive Observational study.

Place and Duration of the Study: University of Port Harcourt Teaching Hospital. January to June 2015.

Methodology: Cytokines were assayed using standard ELISA techniques. Cytokine concentrations were compared across multiple groups by use of the Kruskal-Wallis and Dunn's posts' tests.

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Results: There was a significantly elevated ($p < 00.05$) TNF- α concentration in women with yeast colonization (25.6 pg/ml) when compared to women with normal microbiota (22.2 pg/ml). There was also significant elevated level ($p < 00.05$) of average IL-10 concentrations in women with yeast infection (20.9 pg/ml) when compared to women with normal microbiota (14.8 pg/ml). However, there was no significant difference ($p > 00.05$) in the average IL-2 concentrations of women with yeast colonization and normal microbiota (10.4 pg/ml and 10.2 pg/ml respectively).

Conclusion: The study showed a significant pro-inflammatory immune response to yeast colonization, with a minimal humoral mediated immune response and no hypersensitivity. The local expression of TNF- α and IL-10 increases with the extent of yeast cell colonization.

Keywords: Cytokines; TNF- α ; IL-2; IL-10; yeast; candidiasis.

1. INTRODUCTION

It has been reported that candidiasis could lead to complications in female reproductive health, causing a high socio-economic burden [1]. Yeast colonization by *Candida* sp is the major cause of vulvovaginal candidiasis, which is a common opportunistic fungal infection [2]. While establishing *Candida* sp as one of the infective causes of vaginitis, it is also well noted this fungal organism exist as normal endogenous vaginal microbiota [2,3].

The expression of cytokines are induced by specific stimuli, causing differentiation of many cell types, leading to a complex network of activities that may ultimately eradicate the microorganisms [4,5]. The phenotype of T-helper (Th) cells generated in response to antigenic invasion determines the cytokines produced, course of inflammation and antigen elimination. Th-1 cytokines, including interleukin-2 (IL-2), interferon-gamma (IFN-gamma) and Tumor Necrosis Factor alpha (TNF- α) enhance inflammatory responses and promote a cell-mediated immunity [6]. While Th-2 type cytokines which include IL-4, IL-10 and IL-13 promote humoral immune responses to invading pathogens.

The study was carried out to assess the local concentration of IL-2, IL-10 and TNF- α in vaginal secretions of women presenting with yeast colonization.

2. METHODOLOGY

2.1 Study Population

The study population consisted of women presenting with abnormal vaginal discharge at the clinical consult of the medical microbiology unit of the University of Port Harcourt Teaching

Hospital (UPTH), Rivers state, Nigeria. The study was carried out within a six-month period (January to June, 2015).

2.2 Ethical Consideration

Ethical approval was obtained from the Ethics Committee of the University of Port Harcourt Teaching Hospital. Informed consent was obtained from the patients.

2.3 Specimen Collection and Processing

Dacron coated swabs sticks were used to collect two high vaginal swab (HVS) from each patient presenting with abnormal vaginal discharge at the clinical unit of the medical microbiology department. One of the swabs was used for microscopic analysis and the other swab was placed in 3 mL of phosphate buffered saline (PBS) and centrifuged at 800 g for 10 minutes. The supernatant was aspirated and stored in aliquots at -20°C for subsequent analysis by enzyme linked immunosorbent assay (ELISA) as described by Stute et al. [7].

2.4 Microscopic Analysis of Specimen

Wet mounts of the samples were made to identify budding yeast cells. The wet mounts of each specimen were prepared and viewed under the x40 objective as described [8,9]. The samples were separated into two groups based on their microscopic characteristics as either normal microbiota based on Nugent criterion (Nugent score between 0 – 4, with no clue cells) and yeast slides with budding yeast cells were classified as yeast colonized.

2.5 Cytokine Assay

TNF- α , IL-10 and IL-2 were assayed and quantified using ELISA (Avivabio Systems, CA,

USA), following the manufacturer's instruction. All specimens and controls were run in duplicate. The plates were read at 450 nm, using an ELISA reader (Dynatech Laboratories, Chantilly, VA). The average of duplicates of standards of known concentration were used to generate a standard curve. Cytokine concentrations were determined with corresponding optical densities on the standard curves.

2.6 Statistical Analysis

Cytokine concentrations were compared across multiple groups by use of the Kruskal-Wallis and Dunn's posts' tests. All statistical tests were performed at a 5% significance ($p < 0.05$) using the Prism software package (Graphpad Software Version 6.0).

3. RESULTS AND DISCUSSION

Twenty nine (60.4%) of the subjects had normal vaginal microbiota, while 19 (39.6%) had yeast colonization. The concentration of TNF- α was very high in women with normal microbiota and yeast colonization (22.2 ± 1.4 and 25.6 ± 1.6 pg/ml respectively), followed by IL-10 with a mean concentration of 20.9 ± 1.4 pg/ml in women with yeast colonization, and a mean concentration of 14.8 ± 1.5 pg/ml in women with normal microbiota. The IL-2 concentrations were the lowest of the cytokines, with a mean concentration of 10.4 ± 0.7 pg/ml in women with yeast colonization and 10.2 ± 0.5 pg/ml in women with normal microbiota.

A column scatter plot of the IL-2 concentrations of women with normal microbiota and yeast colonization showed no significant difference (Fig. 1).

A column scatter plot of the IL-10 concentrations of women with normal microbiota and yeast colonization showed a significant difference in cytokine concentrations (Fig. 2).

A column scatter plot of the TNF- α concentrations of women with normal microbiota and yeast colonization showed a significant difference between both groups (Fig. 3).

The findings of this study are slightly in contrast to the findings of a similar study by Hashim et al. which reported a mean IL-10 level of 12.02 pg/ml and a mean TNF- α level of 50.03 pg/ml in vaginal swabs of women with yeast colonization [10]. The mean IL-2 and TNF- α concentrations recorded in study are also in contrast to the

findings of a similar study by Anton et al. [11] which reported mean IL-2 concentration of 9.8 pg/ml and a mean TNF- α concentration of 10.2 pg/ml, with no significant differences between IL-2 and TNF- α concentrations. Bogavac et al. [12] also reported a mean IL-2 level of 12.02 pg/ml and a mean TNF- α level of 50.03 pg/ml in vaginal swabs of women with candidiasis, which are in contrast with the mean IL-2 and TNF- α levels recorded in this study.

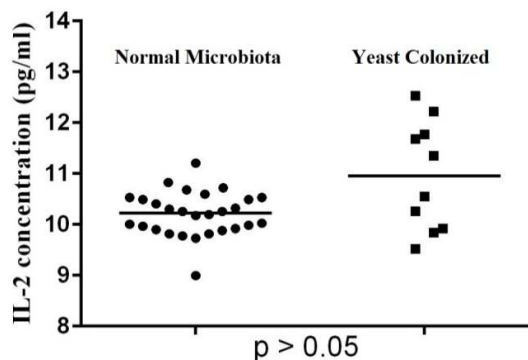


Fig. 1. Column scatter plot of IL-2 concentrations in vaginal swabs of women with normal microbiota and yeast colonization

Solid lines indicate median values for each group. Cytokine levels were compared across groups by use of Kruskal-Wallis test. There is no statistically significant difference ($p > 0.05$) between the cytokine concentrations of both groups

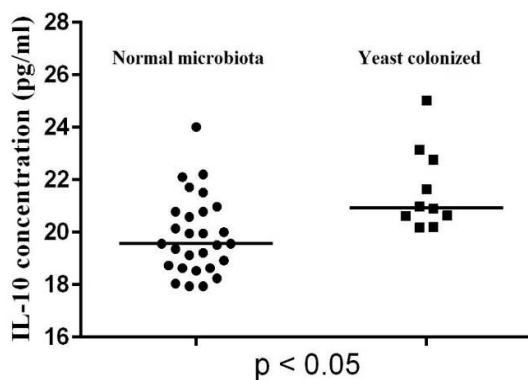


Fig. 2. Column scatter plot of IL-10 concentrations in vaginal swabs of women with normal microbiota and yeast colonization

Solid lines indicate median values for each group. Cytokine levels were compared across groups by use of Kruskal-Wallis test. There is a statistically significant difference ($p < 0.05$) between the cytokine concentrations of both groups

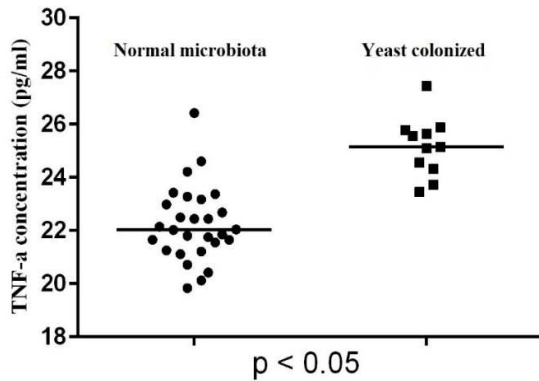


Fig. 3. Column scatter plot of TNF- α concentrations in vaginal swabs of women with normal microbiota and yeast colonization

Solid lines indicate median values for each group. Cytokine levels were compared across groups by use of Kruskal-Wallis test. There is a statistically significant difference ($p < 0.05$) between the cytokine concentrations of both groups

IL-10 concentration were significantly higher in women with yeast colonisation. The increase in IL-10 corresponds with an up-regulation of TNF- α in women with yeast colonization. This primarily anti-inflammatory cytokine dampens the local inflammation, preventing an exaggerated inflammatory reaction [5,6].

The relatively lower TNF- α concentrations observed in this study may also be due to the severity of yeast colonization when compared to that observed in similar studies. This suggests that TNF- α levels increase as yeast colonization increase/progresses in the individual.

Cell mediated immunity by T helper (Th1), type responses is generally considered to be associated with resistance to mucosal candidiasis [13]. The cytokine TNF- α , mediates pro-inflammatory immune responses at the vagina by upregulating leukocyte adhesion molecules on the endothelial cells. TNF- α also serves as a chemoattractant for neutrophils, neutralizing the invading pathogens as reported by Jhun et al. [14].

According to Liao et al. [15] the expression and secretion of IL-2 is tightly regulated in response to antigenic stimulation, inducing macrophage activation and a delayed type hypersensitivity. IL-2 may exhibit pleiotropic functions in mounting immune responses and dampen them when they tend to be exaggerated [16]. There was a slightly

elevated (though non-significant) of IL-2 observed in women with yeast colonization. This suggests a non-hypersensitive immune reaction to the presence of yeast cells. This is most likely attributed to the relatively low severity of yeast colonization.

4. CONCLUSION

There is a significant local pro-inflammatory immune response which may lead to the reduction of invasive pathogenic yeast such as *Candida* sp. The study shows that Th-1 type cytokines are elevated during yeast cell colonization of the genital tract. The elevated levels of TNF- α and IL-10 is a natural immune response to yeast colonization and could prevent a systemic spread of the infection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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