A comparative study of *Lactobacillus* species and inflammatory biomarkers among primigravida and multigravida women

Kushal Gandhi[1], Paula Gutierrez[1, 2], John Garza[1,2], Michael Galloway[1], and Gary Ventolini[1*]

[1] School of Medicine, Texas Tech University Health Sciences Center, TX, USA

[2] The University of Texas of the Permian Basin, Odessa, TX, USA

[*]Principal Investigator

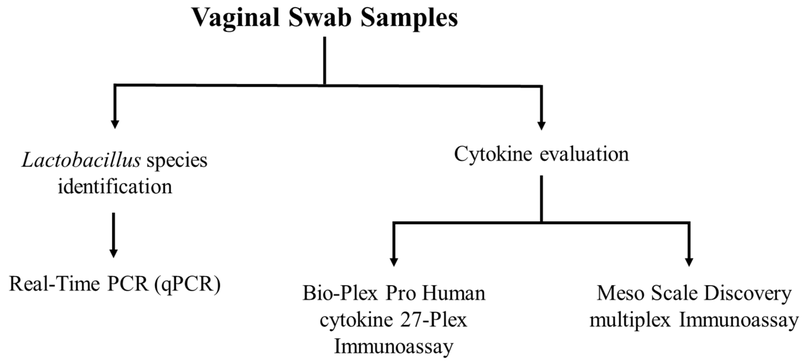
INTRODUCTION

- *Lactobacillus (LB)* species play a vital role in the vagina by inhibiting the growth of pathogenic bacteria through the production of hydrogen peroxide, ribosomal-produced antimicrobial peptides, and lactic acid. *LB* spp. prevalence varies by ethnicity/race, and biographical locations.
- *LB* spp. also aid in the immune defense of the vaginal microenvironment by competitively binding to receptors on the epithelial cells in the vagina. This prevents binding of pathogenic microorganisms to these receptors, thereby preventing activation of the immune response and reducing secretion of pro-inflammatory cytokines. *LB* species in the vaginal microflora vary with *L. crispatus* being the predominant species of Lactobacilli in pregnant women with *L. iners*, *L. Jensenii* and *L. helveticus* following. In pregnant women, *L. crispatus* was shown and correlated with vaginal microbiota stability and a reduced risk of bacterial vaginosis. Pregnant women colonized with *L. gasseri*, *L. vaginalis* or *L. iners* were associated with a higher risk of bacterial vaginosis.
- Cytokines serve as chemical messengers relating to the immune system. There are noticeable differences in the cytokines produced from non-pregnant women versus pregnant women. A study found IL-6 and IL-8 was produced more often in nonpregnant women than in pregnany women. Cytokine levels also depends on the lactobacilli level. Vaginal IL-1B was higher in women whose lactobacilli levels were lower. In women in their first trimester and during labor, IL-1B was associated with a higher risk of bacterial vaginosis which has shown to correlate with adverse pregnancy outcomes.
- Pregnancy is associated with changes to the vaginal microenvironment secondary to both physiologic and behavioral factors, which results in alterations to the balance of cytokines and a change in the overall immune response. It is surmised that proper regulation of the inflammatory response in pregnancy is vital to proper tolerogenesis of the fetus and an overall healthy pregnancy.

OBJECTIVES

The objective of the study is to identify the vaginal lactobacilli profile and to determine inflammatory biomarkers (cytokine) among primigravida (PG) and multigravida (MG) women.

MATERIALS AND METHODS



RESULTS

		Primigravida	Multigravida
Maternal Age (years)	Average	31	41
	Range	19-65	22-78
Parity	Average	1	3
	Range	---	2-7

Table 1: Characteristics of women included in the study. Age and parity of patients at the time samples were collected.

Cytokine	Primigravida (m, pg/ml)	Multigravida (m, pg/ml)	Δm	Mann- Whitney U Test (*p*)
IP-10	9.730	81.610	+71.88	0.016*
IL-10	0.3350	0.835	+0.500	0.126
IL-2	0.4800	0.400	-0.080	0.247
MIP-1α	0.0000	0.170	+0.170	0.273
IL-13	2.0385	2.250	+0.212	0.296
IL-1β	7.0300	2.305	-4.725	0.335
PDGF-BB	32.42	19.84	-12.58	0.360
IL-4	0.060	0.070	+0.010	0.394

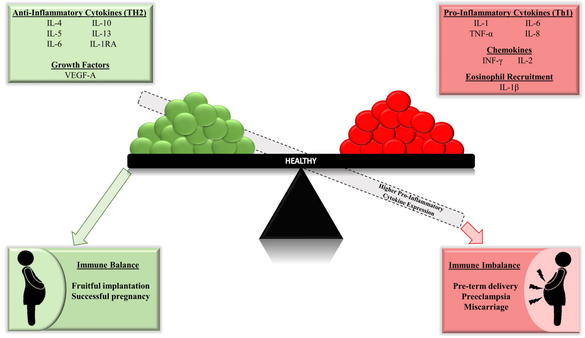
Table 2: Cytokine levels in primigravida and multigravida women. Data are from independent samples with *p*-values calculated using the Mann-Whitney U test with two-sided alternative hypothesis. (m, median conc. of cytokines measured in pg/ml)

Lactobacillus spp.	*Lactobacillus* spp. identified in number of women		Percentage of women with *Lactobacillus* spp. identified (%)		Total number of women (*n*)	
	Primigravida	Multigravida	Primigravida	Multigravida	Primigravida	Multigravida
L. crispatus	13	15	36 %	23 %	36	66
L. jensenii	1	0	3 %	0 %		
L. gasseri	4	9	11 %	14 %		
L. iners	10	22	28 %	33 %		

Table 3: *Lactobacillus* species identified in primigravida and multigravida women. *Lactobacillus* species abundance as determined by qPCR analysis.

DISCUSSION

- Vaginal cytokines and the *Lactobacillus* species responsible for their production are of fundamental interest in obstetrical and perinatal medicine due to the vital role that inflammation plays in a healthy and successful pregnancy and birth. The current results indicate that *L. crispatus* was the dominant species in primigravida women, while *L. iners* was the most abundant species in multigravida women. *Lactobacillus* spp. are miscellaneous, and their distribution is diverse based on race, residential area, and disease state[1].
- The protective mechanisms of *Lactobacilli*, include maintaining an acidic vaginal pH and the production of antimicrobial products such as cytokines and hydrogen peroxide which facilitate a healthy environment for pregnancy. The inflammatory dysregulation in response to infection, oxidative stress, and other factors. An imbalance of the cytokine network is implicated in insufficient tolerogenesis in the fetus and may lead to adverse pregnancy outcomes such as miscarriage and PE[2].
- Our study focused on vaginal cytokines, since they are considered a better source of biomarkers in predicting PTB, PE and other obstetrical conditions when compared to plasma or serum cytokines. While serum cytokines may be a useful index to define the etiology, owing to the fact that they are a mixture of secretions from gestational and reproductive tissues[3].



CONCLUSION

There were significant differences regarding cytokines, chemokines and *Lactobacillus* species among four groups of studied patients. *L. crispatus* was the most abundant in primigravida. The multigravida group had significantly higher levels of *L. iners* and IP-10. Additionally, the presence of *L. gasseri*, *L. iners*, and *L. crispatus* was associated with the levels of MIP-1β, TNF-α, IP-10, VEGF-A, IL-10, and IL-1β in the vagina. In the future, these results will be a vital foundation in the search for vaginal biomarkers in pregnancy.

REFERENCES

1. Mastromarino P, Vitali B, Mosca L. Bacterial vaginosis: a review on clinical trials with probiotics. New Microbiol. 2013;36(3):229-38.
2. Yeh CC, Chao KC, Huang SJ. Innate immunity, decidual cells, and preeclampsia. Reprod Sci. 2013;20(4):339-53.
3. Buxton MA, Meraz-Cruz N, Sanchez BN, Gronlund CJ, Foxman B, Vadillo-Ortega F, et al. Air pollution and inflammation: Findings from concurrent repeated measures of systemic and reproductive tract cytokines during term pregnancy in Mexico City. Sci Total Environ.

ACKNOWLEDGEMENTS

We are grateful to the Texas Tech University Health Sciences Center, Permian Basin, TX and Cathy Lovett, Ailena Mulkey, Evangelina Santiago, and Jammie Holland from Clinical Research Institute, TX for their excellent support on this study. In addition, we would like to acknowledge Dr. Scott Gygax and his team for his assistance in the quantification of the vaginal flora by qPCR analysis.