Collagen Integrity of the Uterine Cervix Reflects Amniotic Fluid Cytokine Profile

Sean M. Keeler, MD[1,3], Daniel Kiefer, MD[2], Orion Rust, MD[3], Nadav Schwartz, MD[1], Matthew Sottile Ph.D.,[4] Carolyn Salafia, MD, MS[5]

[1]New York University Medical Center, Department of Obstetrics & Gynecology, New York, New York, [2]Winthrop University, Department of Obstetrics & Gynecology, Mineola, New York, [3]Lehigh Valley Hospital, Department of Obstetrics & Gynecology, Allentown, Pennsylvania,
[4]Department of Computer and Information Science, University of Oregon, Eugene, OR, [5]Placental Analytics, LLC, Larchmont, NY.

Abstract

OBJECTIVE: To determine if there is a correlation between the histological staining characteristics of cervical collagen and amniotic fluid cytokines in women with an ultrasonographic short cervix in the midtrimester.

STUDY DESIGN: Asymptomatic women with a transvaginal cervical length ≤ 25mm between 16 - 24 weeks underwent a micro-cervical biopsy and amniocentesis. Amniotic fluid cytokine concentrations were assayed using the Bio-Plex multianalyte detection and quantitation system (Bio-Rad, Hercules, CA.).Cervical biopsy specimens were stained with hematoxylin and eosin (H&E) and examined by a single reviewer blinded to clinical and assay data. One 40X photomicrograph was taken of each biopsy. The R channel histogram was extracted and its mean, standard deviation, skew and kurtosis calculated. A single collagen staining factor score (CFS) was extracted from these data by principal components analysis. Spearman's correlation was used to compare the CFS to levels of amniotic fluid (AF) cytokines.

RESULTS: Thirty three paired AF and micro-cervical biopsy specimens were available for analysis. Spearman's correlations demonstrated associations of the CFS with AF IL-6, IL-8, Eotaxin, IP-10 and MCP-1 (each $p < 0.05$).

CONCLUSION: Image segmentation allows extraction of collagen structural features that are highly correlated with inflammatory cytokines, suggesting that intra-amniotic inflammation and the structural integrity of the cervix are related. Further study may help elucidate the temporal relationship between collagen integrity and inflammation, allowing for pathway specific therapy.

Background

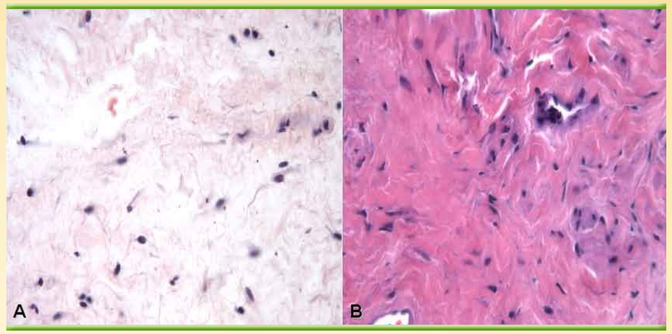
- Cervical ripening is an active cellular process of metabolism and reorganizing of the collagen matrix.
- Inflammation plays a central role in initiating the cervical remodeling that ultimately leads to spontaneous preterm parturition.
- IL-6, IL-8 and Monocyte chemotactic protein-1 (MCP-1) are increased in cervical tissue of patients who are in preterm labor.
- Pro-inflammatory cytokines, which are exceedingly elevated in spontaneous preterm deliveries, mediate matrix metalloproteinase, arachidonic acid and prostaglandin production.
- The inflammatory cascade causes collagen degradation, remodeling, and impaired collagen integrity.
- We sought to determine if there is a correlation between the histological staining characteristics of cervical collagen and amniotic fluid cytokines in asymptomatic women with an ultrasonographic short cervix (≤ 25mm) in the midtrimester.

Materials and Methods

- All studies were performed under an Institutional Review Board approved protocol.
- Inclusion Criteria
  - All pregnancies between 16 - 24 weeks' gestation.
  - Ultrasound-diagnosed cervical length ≤ 25mm.
  - Funneling of chorio-amniotic membranes ≥ 25% into the endocervical canal.
- Exclusion Criteria
  - Any known fetal chromosomal or structural anomaly
  - Ruptured membranes
  - Vaginal bleeding
  - Prolapse of endocervical membranes beyond the external cervical os
  - Persistent uterine activity accompanied by cervical change
  - Obstetrically indicated delivery.
- Patients were consented for a controlled fine needle biopsy (FNB) of the uterine cervix.
  - The Bard Monopty® biopsy instrument (C.R. Bard, Inc., Murray Hill, N.J.),
    - Automated spring-loaded instrument that extends an 18 gauge cutting needle to penetrate 11mm into the cervical stroma.
    - Obtains a 7mm x 0.003cm^3 controlled FNB.
- All patients had an ultrasound-guided transabdominal amniocentesis
  - Evaluation for intra-amniotic infection (low glucose, elevated WBC count, and aerobic/anaerobic culture)
- Five milliliters (5ml) of unspun amniotic fluid was aliquoted into 15 ml polypropylene tubes
  - Stored at -70ºC for future cytokine analysis
- Amniotic fluid samples were simultaneously analyzed for 25 inflammatory mediators using the Bio-PlexTM array system (Bio-Rad Laboratories Inc, Hercules, CA)

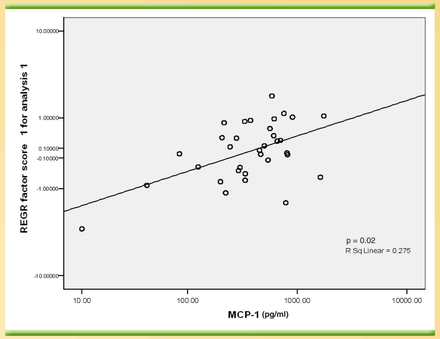
- Interleukin (IL)-1β	- Eotaxin
- IL -1ra	- Granulocyte colony stimulating factor (G-CSF)
- IL -2	- Interferon gamma (IFN-γ)
- IL -4	- Inducible protein-10 (IP-10)
- IL -5	- Monocyte chemotactic protein-1 (MCP-1)
- IL -6	- Macrophage inflammatory protein
- IL -7	(MIP)–1a (MIP-1a)
- IL -8	- MIP-1b
- IL -9	- Platelet derived growth factor (PDGF) bb,
- IL -10	- Tumor Necrosis factor alpha (TNF-α)
- IL -12	- Regulated on activation normal, T cell expressed and
- IL -13	secreted (RANTES)
- IL -15	- Vascular endothelial growth factor (VEGF)
- IL -17	

- Cervical biopsy specimens were stained with hematoxylin and eosin (H&E)
  - Examined by a single reviewer blinded to clinical and assay data.
  - 40X photomicrograph was taken of each biopsy.
    - Collagen properties were characterized based on the distribution of intensities in the red color channel of the images.
    - The level of red was quantified by measuring the distribution of pixel intensities (zero to 255)isolated in the red channel.
    - R channel histogram of pixel intensities was extracted.
    - Mean, standard deviation, skew and kurtosis calculated.
  - A single collagen staining factor score (CFS) was extracted from these data by principal components analysis.
    - High CFS represent poor collagen integrity.
      - The biopsy image is composed of loose, poorly interconnected collagen fibrils, thus has alot of white space in the image.
    - Low CFS represent good collagen integrity.
      - The biopsy image is composed of dense, closely interconnected collagen fibrils, thus has little white space in the image.
  - Spearman's correlation was used to compare the CFS to levels of amniotic fluid (AF) cytokines



A. High CFS cervical biopsy at 22wks. Patient delivered preterm (30 wks).

B. Low CFS cervical biopsy at 23 wks. Patient delivered at term (38wks).



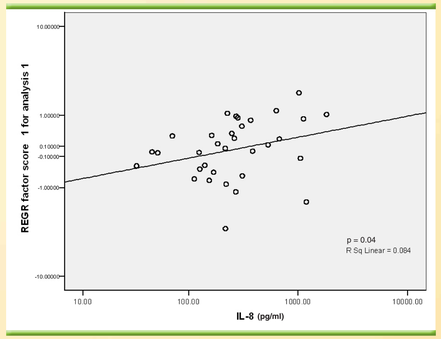
Results

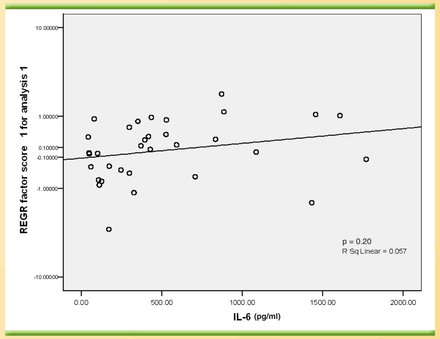
Table 1. Study characteristics

Characteristic	N = 33
Age (years)	25.3 ± 6.3
Race	
Caucasian	13 (39.4)
Hispanic	13 (39.4)
African American	6 (18.2)
Asian	1 (3.0)
Multiparity	16 (48.5)
Singleton gestation	28 (84.8)
Previous spontaneous preterm birth	17 (51.5)
Earliest spontaneous preterm birth (weeks)	25.3 ± 5.9
Cervical length at enrollment (mm)	14 ± 6.8
Fetal fibronectin + *	7 (21.2)

Table 2. Correlation between histological staining characteristics of cervical collagen and amniotic fluid cytokines

Variable (n = 33)	Correlation Coefficient (r)	P value
IL-6	0.36	0.04
IL-8	0.38	0.03
MCP-1	0.39	0.02
IFN-γ	0.30	0.09
Eotaxin	0.35	0.04
IP-10	0.35	0.05
MIP-1b	0.32	0.07
Cervical length	-0.03	0.78





Conclusion

- Image segmentation allows extraction of collagen structural features that are highly correlated with inflammatory cytokines.
- This suggests that intra-amniotic inflammation and the structural integrity of the cervix are related.

WINTHROP University Hospital

School of Medicine, New York University

A PASSION FOR BETTER MEDICINE.™ Lehigh Valley Health Network

610-402-CARE LVH.org