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Stemness biomarkers in the cervical smear of patients with a mid-trimester short cervix

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Objective

The presence of stem cells has been previously described in human precancerous and malignant cervical cultures. Previous studies have shown a direct interplay of the stem cell niche, which is practically present in every tissue, with the extracellular matrix. In the present study we sought to determine the expression of stemness markers in cytological specimens collected from the ectocervix among women with cervical shortening during the second trimester of pregnancy and women with a normal cervical length.

Methods

This study was approved by the Ethical Review Board of Alexandra General Hospital. All patients who participated in the study provided written informed consent before the procedure. A cervical smear was obtained between 20 and 24 weeks of gestation from 41 women with a cervical length of less than 25 mm (study group) and 17 healthy women with a normal cervical length (control group). Sampling was performed following the same procedure used for liquid-based cytology. Samples were stored at -80°C for further study. Concurrent with the retrieval of the cervical smear a vaginal culture was also taken as this is part of the clinical routine in all patients with cervical insufficiency. To ensure that the presence of vaginal infection would not have an impact on the outcomes of cervical swabs, we retrieved cultures in control women as well and opted to include only pregnant women with normal vaginal flora. RNA was extracted using a commercially available kit (Monarch Total RNA Mini- prep Kit, New England Biolabs Inc), which enables high-throughput purification of total RNA from up to 96 cultured-cell samples using silica-membrane RNeasy 96 plates. The RNA extracted from cervical cells was used to obtain complementary DNA (cDNA) with reverse transcription, using LunaScript RT SuperMix Kit (New England Biolabs). Quantitative Real-time PCR was performed with Light Cycler 480II (Roche Molecular Biochemicals, Mannheim Germany) using the SYBR Luna Universal qPCR Master Mix kit (New England Biolabs Inc). The solution for OCT4, DAZL, Nanog and G6PD as control gene consisted of 10μl Master mix, 1μl of Forward Primer (10pm/μl), 1μl of Reverse Primer 100 (10pm/μl), 3μl H2O. The primers used for this trial were provided by Eurofins Genomics. The primers and the hybridization probes used for this trial were provided by TIB-MOLBIOL. The qRT-PCR reaction cycling profile was 30 sec at 95oC, 1 cycle, 5 sec at 95oC and 30 sec at 60 oC, 40 cycles. The 2-ΔΔCT method, was used to calculate the relative transcript abundance. All qRT-PCR reactions were repeated twice.

Results

The expression of OCT-4 and NANOG was higher in the cervical insufficiency group compared to the control group (-5.03 (-6.27, -3.72) vs -5.81 (-7.67, - 19 5.02) p=.040 for OCT4) and (-7.47 (-8.78, -6.27) vs -8.5 (-10.75, -7.14), p=.035 for NANOG. Differences in the DAZL gene were not significantly different (5.94 (4.82, 7.14) vs 6.98 (5.87, 7.43) p=.097). Pearson correlation analysis indicated the existence of a moderate correlation of OCT-4 and Nanog with cervical length. The findings of our study suggest the presence of increased activity of stemness markers in cervical swab specimens of pregnant women diagnosed with cervical shortening, as their expression was significantly higher compared to that of specimens from control pregnant women. Moreover, there seems to be a direct correlation between the expression of OCT-4 and Nanog and the actual cervical length and even though the predictive accuracy of the model was moderate, the use of random forest analysis permitted optimization of the predictive accuracy of the model.

Conclusion

The findings of our study propose an enhanced activity of stemness biomarkers among pregnant women diagnosed with a short cervix. It remains unknown, if the different expression may trigger the onset of preterm birth or it is a secondary effect that is triggered by the presence of mechanical, proinflammatory and hormonal alterations that occur during pregnancy. Therefore, further studies are needed to evaluate its diagnostic accuracy in samples retrieved during the first trimester of pregnancy and these should ideally focus on patients at increased risk of developing cervical shortening (history of prior preterm birth, second trimester abortion, cervical conization, etc).