Prediction of Cervical Cancer Precursor Lesions by Quantitative Methylation Specific PCR: a Retrospective study

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Introduction

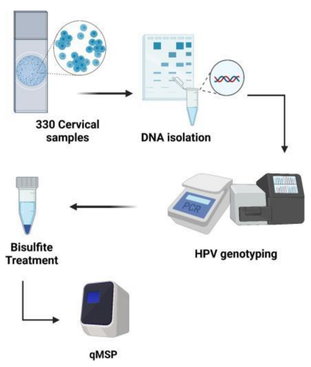
Since the discovery of the Papanicolaou test, several limitations were observed in the cervical cancer screening program. As the infection with a high-risk HPV (hr-HPV) genotype is associated with the development of cervical cancer, hr-HPV-based screening was implemented. However, the specificity of hr-HPV-based screening is low, therefore further triaging is required. DNA methylation is one of the common epigenetic modifications in cervical cancer, which can be used as a triage tool. More than 100 genes were found methylated in cervical cancer. Ten of those genes were found frequently methylated in Cervical Intraepithelial Neoplasia (CIN) 2,3 and cervical cancer one of them FAM19A4 and mir-124-2. Both genes were studied extensively in cervical samples and yielded good results in detecting women who are at risk for cervical cancer development (1)(2)(3).

Objectives

This study was undertaken to evaluate the performance of *FAM19A4* and *hsa-mir-124-2* hypermethylation as a triage tool for women who are at risk of developing cervical cancer or high-grade cervical cancer precursor lesions by taking into consideration the cytology report, histology diagnosis, and human papillomavirus (HPV) status.

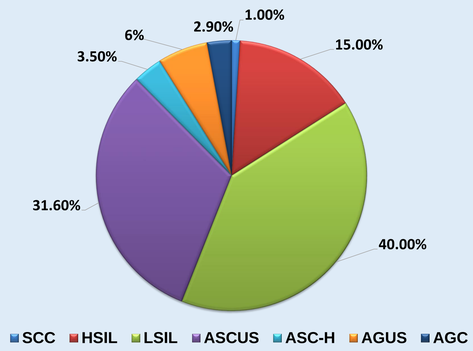
Methods

- DNA was extracted from 330 cervical ThinPrep samples using MagNA Pure LC, which is an automated system.
- HPV DNA in cervical samples was detected by real-time PCR using two different sets of primers MY09/MY11 and Gp5+/Gp6+
- HPV genotypes were identified using Sanger sequencing analysis. The sample results were analyzed using Sequencing Analysis software v 3.7 (Applied Biosystems). The HPV genotypes were identified using BLASTn software (http://www.ncbi.nlm.nih.gov/blast/htlm)
- The DNA extracts were bisulfite-treated using EpiTectFast96 bisulfite conversion kit (QIAGEN,Hilden,Germany). Bisulfite conversion of unmethylated cytosine to uracil was followed by the cleanup of the bisulfite-converted DNA.
- The hypermethylation of FAM19A4 and mir-124-2 genes in cervical samples were detected by qMSP using The QIAsure methylation kit (QIAGEN, Hilden, Germany).
- The data were analyzed using SPSS.

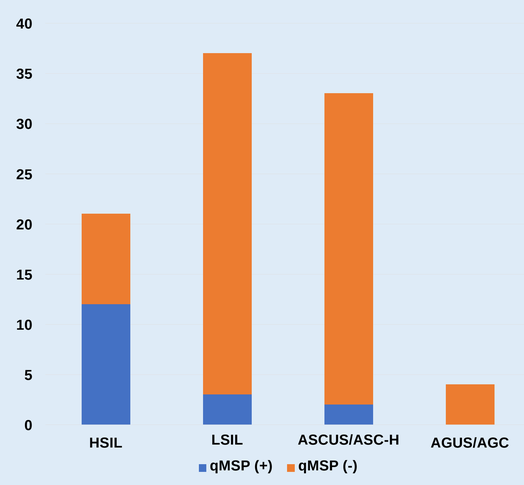


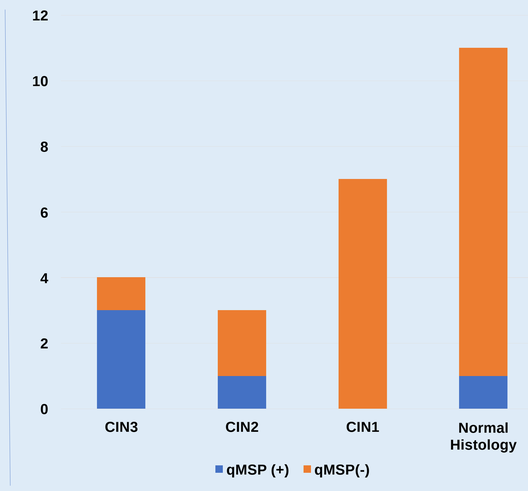
Results and Discussion

- 282 ThinPrep cervical samples with abnormal cytology results were used. The age range of women involved in the study was between 20 and 69 years with a median age of 38 years. All women were non-pregnant at the time of sampling.
- According to the cytology reports of women involved in the study, 3 (1%) cases were cytologically diagnosed with Squamous cell carcinoma (SCC), whereas 42 (15%) cases were diagnosed with High-grade squamous intraepithelial lesion (HSIL). Most cases were diagnosed with Low-grade squamous intraepithelial lesion (LSIL) (n = 113, 40%) or ASCUS (n = 89, 31.6%).

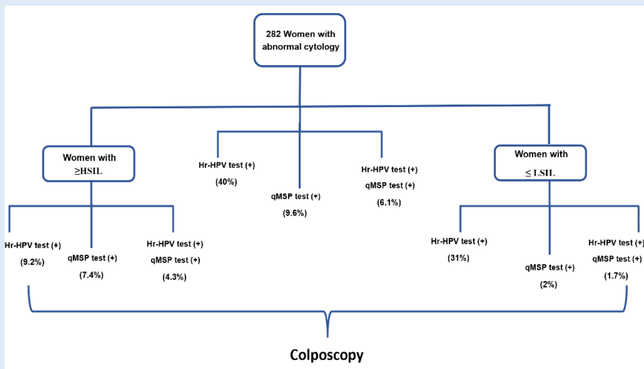


- HPV DNA was detected in 54.3% of the samples (n = 153). Hr-HPV was detected in 26 (86.7%) out of 30 women with HSIL+, 48 (56.5%) out of 85 women with LSIL, 37 (55.2%) out of 67 women with ASCUS/ASC-H, and 4 (50%) out of 8 women with AGUS/AGC. Moreover, hr-HPV was detected in 3 (100%) cases with SCC, 4 (100%) cases with CIN3, 3 (100%) cases with CIN2, 7 (58.3%) cases with CIN1
- Out of 280 samples with abnormal cytology results, 27 (9.6%) samples had a positive qMSP test, while 253 (90.4%) had a negative qMSP test.
- In hr-HPV positive samples with cytology results, 12 (57.1%) women diagnosed with HSIL had hypermethylated FAM19A4/mir-124-2 genes, whereas only 3 (8.1%) with LSIL had positive qMSP test. Only 2 (6%) out of 33 women with ASCUS/ASC-H had positive qMSP test. In women with hr-HPV infection, the risk of being diagnosed with HSIL was increased 23.5 times (95% CI: 7.14-77.66) when FAM19A4/mir-124-2 genes were hypermethylated
- In samples with histology and hr-HPV infection, 75% of women diagnosed with CIN3 had hypermethylated genes, followed by 33.3% of women with CIN2, and 9% of women with normal histology. There was a statistically significant association between being diagnosed with CIN3 and having hypermethylated genes in samples positive for hr-HPV (p = 0.002, Phi coefficient 0.497).





- The sensitivity of the qMSP test to predict histological lesions among women with hr-HPV was 38.9% for CIN1+, 57% for CIN2+, and 75% for CIN3. The specificity of the qMSP test was 91%. Regardless of HPV results, the sensitivity of the qMSP test to predict histological lesions, was 28.6% for CIN1+, 47.4% for CIN2+, and 61.5% for CIN3, while the specificitywas 95.3%.
- Currently, the protocol followed in the maternity hospital is that if the woman was primarily diagnosed with cytology abnormal lesions and had positive hr-HPV results, she will be referred for colposcopy examination (Personal communication). Applying this protocol to this study, 115 (40%) women with hr-HPV results and abnormal cytology will be referred to colposcopy. However, using cytology and qMSP test results only, will reduce the referral number to 27 (9.6%) women, which reduces around (31%) of the colposcopy referral rate. Noteworthy, 21 (78%) out of 27 women with methylation positive tests were diagnosed with HSIL.



Conclusion

This study supports previous studies in which FAM19A4/mir124-2 methylation assay can be used as a safe triage tool to differentiate between women at risk for cervical cancer progression which requires immediate treatment, from women with lower-risk lesions that have a high chance for regression, which will reduce the colposcopy referral rate. Future work is suggested to be done on triaging women with lower-grade lesions using methylation analysis, which will further reduce the colposcopy referral rate and prevent women from overtreatment.

Acknowledgments

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