Cervix cancer screening and prevalence of HPV in Mont Ngufola health district/ Kinshasa/ DR Congo.

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on behalf of the women profile for Africa
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Introduction
Cervical cancer is a leading cause of death in women in Africa, with high incidence and mortality rates in the Democratic Republic of Congo (DRC).

The women profile for Africa project aimed to:
- estimate the validity of screening tools,
- estimate the prevalence of HPV infection and HPV-genotypes
- assess the correlation between HPV and cervical cytology.
- pilot a web-based cancer registry.
- Understand community perception and perspectives.
- Comparing performance on screening tools between gynaecologists versus MD versus nurses, Pathologists versus lab technicians
Deaths related to cancer in women from DRC: 2014
Source: DRC MoH
Studies done in DRC


Several attempts have been done to start screening and/or sensibilisation activities on cervical cancer since 1992.

Studies achieved mainly in specific group as HIV population

Recommendations formulated:
- VIA et VILI but results on sensitivity are largely different from country to country but also from different studies.
- Pap-tests. WHO does not recommend Pap-test in developing countries.
- HPV strains are different from the strains in Europe and North America although HPV 18 was identified in 33% of 54 HIV infected patients stating that vaccination could be problematic if targetted strains are discordant.

Until 2014 still no screening or vaccination program that has been initiated in DRC.

Cervical Cancer is mostly identified at a late stage with a high mortality.
Methodology
Validation of screening tools

- Training on VIA, VILI, Pap test collection and reading, urines collection, Colposcopy for MD, nurses, pathologists, gynecologists was done by experts in specific field from Italy

- We recruited women for 30 years old to 50 years old.

- We performed VIA, VILI (two times by a MD and a nurse), Pap tests to all recruited women

- Colposcopy and biopsy were performed on positive on either VIA, VILI or Pap test and also on a sample of negative in all screening tools.

- Bethesda system was used to classify cervical samples

- Biopsy is used as a gold standard

- Control quality was performed on all pap tests and biopsy by cytologist in Italy.
We use dried urine spots (DUS) samples

HPV-DNA was detected by nested-PCR (ORF L1) and infecting genotypes through RFLP technique.
Collection of **cervical samples** is not always easy
- in resource-limited settings
- in populations where these procedures may be less well accepted (ex. for young age or socio-cultural/religious implications)

**Urine sample** for the detection of HPV infection
→ non-invasive
→ more accessible and acceptable to women
→ less expensive
→ bypasses medical examination
→ even easier to perform than self-collected vaginal swabs

consequently, the screening coverage could be increased primarily by reaching populations in less developed regions
HPV Testing from Dried Urine Spots (DUS)

DRC HEALTHCARE FACILITIES

• 50μL of urine samples were spotted on 5 preprinted circles on a filter paper
• DUS was dried for 3h
• DUS was stored in a paper bag in a dry place at RT (25–30°C)

Urine samples collection and DUS preparation
November 2014 - January 2015
N = 456
Asymptomatic women, 30-49 years of age
• 3 circles were cut out using a sterile scalpel blade, transferred into 1mL of Lysis Buffer, incubated on a roller mixer for 30’ at RT and then centrifuged for 15’’ at 1500 ×g
• **Nucleic acids extraction**: lysate (750μL) was extracted using the NucliSens EasyMAG method
• **HPV detection**: nested PCR amplifying a fragment of 150 bp of **ORF L1** region
• **HPV genotyping**: first step (450 bp) of HPV positive samples was genotyped using RFLP technique

**HPV detection and genotyping (31/03/2015)**

N = 242/456 (53%)

(all DUS prepared in November-December 2014)
Community perception and perceptives

- We used:
  - focus group discussions
  - Interviews of health workers
Preliminary results
Validation of screening tools

n=864 with age range from 30 to 50 years old.

<table>
<thead>
<tr>
<th></th>
<th>positive</th>
<th>negative</th>
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<tbody>
<tr>
<td>VIA</td>
<td>65 (8%)</td>
<td>747 (92%)</td>
</tr>
<tr>
<td>VILI</td>
<td>104 (12.1%)</td>
<td>759 (87.9%)</td>
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<tr>
<td>VIA+VILI*</td>
<td>143 (16.6%)</td>
<td>721 (83.4%)</td>
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</table>

* = positive women on either test (either VIA or VILI positive)
<table>
<thead>
<tr>
<th>PAP</th>
<th>VIA_VILI (POSITIF)</th>
<th>VIA_VILI (NEGATIF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical cellular reactive (ACR)</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>111</td>
<td>617</td>
</tr>
<tr>
<td>Atypical Glandular Cells (AGC)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>atypical squamous cells of undetermined significance ASCUS</td>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td>atypical squamous cells cannot exclude HSIL ASC-H</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>LSIL</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>HSIL</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Squamous cells carcinoma</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>143 (16.6)</td>
<td>721 (83.4)</td>
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</table>
Determination of HPV strains testing from urine and Dried Urine Spots (DUS)

HPV Prevalence: 121/242 (50%)

<table>
<thead>
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<th>HPV: IARC 2011 classification</th>
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<tr>
<td>HR Clade</td>
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<tr>
<td>LR</td>
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HPV Genotype distribution

Total number of infections: 171
Correlation between HPV and cytology

During November 2014–February 2015, DUS and cervical samples were collected from 209 women (median age, 37.2y).

HPV–DNA prevalence was 56.5% (IC95% 49.7–63).

Overall, 167 infections and 39 genotypes were detected.

73.6% (IC95% 66.5–79.7) of all infections was sustained by at least one genotype of HR–clade,

HPV–26 (8.4%) and HPV–16 (6%) were the prevalent.
Correlation between HPV and cytology

- As for cytology results,
  - 32% resulted inadequate,
  - 48.3% negative,
  - 19.7% positive (6.7% ASC-us, 7.2% L-SIL, 2.9% ASC-H, 2.9% H-SIL).

- The prevalence of HR-HPV infections increased with abnormal cervical cytology result:
  - 30.7% in negative
  - and 35.7%, 40%, 50%, 83.3% in ASC-us, L-SIL, ASC-H, H-SIL respectively.
Perception and barriers for cancer screening program

1. Economical barrier

• Poverty
  • Fees for a consultation: 25 USD, Transport fees to reach the centre: 3 USD, Fees for screening tests and collection of pap samples: 10 USD, Fees for pap test reader: 30-50 USD. (lack of cytotech.), Fees for biopsy if needed: around 250 USD. Treatment: ? Depend on the level of lesion. Chemotherapy and radiotherapy can reach 2000 or 3000 USD per course.

Resolution: providing free access to screening? But How and by who??

• Opportunity lost: Women who survive daily with small trade do not adhere because they perceive it as loss of income during the time they are at the screening facility.

«...who will sell for me? I cannot abandon my table...»

. Treatment cost for precancerous lesions but also for cancer because at the beginning of screening certainly some invasive cancers will be detected. Chemotherapy and radiotherapy are not affordable in DRC and there is no mutual or health insurance.
2. Geographical barriers

• Screening facilities need to be accessible and as close as possible to the population.

  • *Problem*: service not available for women.

  • *Solution*: Set up 4 pilot screening centers in Kinshasa with Pap test as screening tool.

  • But in rural area, facilities are rare and we need to think how to solve this problem.
3. Cultural barriers

Lack of information on cervical cancer screening: women continue to be ignorant and don't seek correct information

Habits: In general, women don't consult when they are not feeling ill. Their husband cannot let them to see a gynecologist when they are not seek. Problem of privacy.

Ashamed to have an examination when not ill, even the husband does not accept that his wife goes to a consultation when she's not ill.

Fear of knowing that they have cancer « I want to die if I find out that I have cancer »

Beliefs: The « bad » illness = bad luck related to bad spirits « God cannot permit that this illness comes into my family » « Accept screening is opening the door for the devil to enter our family »
What these preliminary results suggest?

✓ Pre-cancerous lesions are frequent in women between 30 and 50 years

✓ Validation tools are ongoing but pap-tests seems the best with the possibility of remote quality control. Its feasibility and its costs (30-50 US dollars for readers) in remote area is a limitation. The is a need of cyto-technicians in DRC in support of pathologist.

✓ HPV prevalence (50%) is high, but not so far from that observed in studies conducted in Africa using the same molecular methods in women with normal cytology (Kenya: about 40%, Mozambique: 32-41%)

✓ The genotype distribution is similar to that found in women of other regions, including those in developed countries (and Italy)
More than 60% of HPV-DNA positive women is infected with at least one genotype of HR-clade, about 40% with HR genotypes of Group 1.

These data, although preliminary, support the need for prevention interventions targeted at women of this age group (> 30 yrs).

DUS could be a useful tool for planning cervical cancer screening strategies, especially in less developed regions.

DUS could also be useful for:
- Epidemiological/virological surveillance where pelvic examination is not practical (ex. post-vaccination surveillance in adolescent women) or where other strategies are difficult to apply.
- Monitoring of type-specific prevalence (vaccine-preventable HPV types - other non-vaccine types).
Based on generated data and evidence, we will build a rationale, effective and efficient algorithm for cervix cancer screening in DRC combining HPV test, pap test, VIA, VILI and having in mind the rural remote area.

but according to the prevalence, **colposcopy** can become a real bottleneck in case of VIA or HPV testing with this prevalence,
- too many colposcopy will cost more and will be impossible due to
  - lack of colposcopes
  - and trained colposcopists.
Finally, we forgot to have a PhD student on this project.

Thanks for your attention.