Cohort Profile: African Collaborative Center for Microbiome and Genomics Research’s (ACCME’s) Human Papillomavirus (HPV) and Cervical Cancer Study

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Why was the cohort set up?

Globally, cervical cancer is the fourth most common cancer among women, with an estimated 528 000 new cases in 2012.1 Although it remains a significant public health problem worldwide, the burden of cervical cancer falls disproportionately on low-resource countries. In the USA, the incidence rate of cervical cancer was 6.6 per 100 000 in 2012,1 compared with 23.0 per 100 000 in Nigeria2 which had about half the population of the USA in 2012.

Persistent high-risk human papillomavirus (hrHPV) infection of the cervix is a necessary cause of cervical cancer.3,4 About 80% of sexually active individuals become
infected with at least one genital hrHPV in their lifetime. Most of the infections are cleared, but about 10% become persistent infections. \(^5\)-\(^7\) The mechanism by which some individuals are able to remain uninfected while some clear the infection and others remain persistently infected, remains unclear. Of the women with persistent hrHPV infection, only \(~ 12\%\) go on to develop cervical intraepithelial neoplasia (CIN)\(^2\),\(^3\) and cervical cancer.\(^8\) Therefore, several co-factors are required to support the cervical carcinogenesis induced by persistent hrHPV infection.\(^7\),\(^9\)

Data from Western series suggest that HPV types 16 and 18 account for most of the cases of cervical cancer in those environments.\(^4\) However, there have been few longitudinal studies of hrHPV infections and their association with cervical cancer in much of the rest of the world. Given the marked heterogeneity of types and prevalence of multiple hrHPV infection in many parts of the developing world, variation in ability of different hrHPV types to establish persistent infection and concerns about the coverage of existing vaccines, longitudinal studies of large numbers of women in the general population in different regions of the world, with information on HPV types and risk factors, are critical to bridging the knowledge gaps, especially in African populations where such studies are scarce.\(^10\)-\(^14\)

The aetiology of cervical cancer is multifactorial. It is clear that environmental risk factors such as smoking, age at first sexual intercourse, age at first full-term pregnancy, high parity and use of oral contraceptives which have been implicated in persistent hrHPV infection, do not completely explain the association between hrHPV infection and cervical cancer, and several studies have identified a role for genetics and heritability in its aetiology.\(^15\)-\(^23\) These studies suggest that genetic factors contribute to the risk of persistent hrHPV infection and progression to cervical cancer. A recent review of 15 studies on heritability of cervical cancer risk suggested that having a first-degree relative with cervical cancer increases an individual’s risk by 1- or 2-fold.\(^24\) Other reviews concluded that there is a potential role for genetic factors in cervical cancer \textit{in situ} and estimated the heritability to be between 11\% and 15\%.\(^25\)-\(^27\) Nevertheless, there have been few studies of genetic risks of the different components of the pathway to cervical carcinogenesis among all populations, particularly in Africans.

An important yet understudied aspect of HPV carcinogenesis is the role of innate immunity and emerging knowledge about the vaginal microbiome, and the role that these play in cervical carcinogenesis.\(^28\)-\(^34\) Vaginal microbiota may affect the risk of persistent hrHPV infection through rich, complex, dynamic and individual-specific microbial interaction with the host such as through signaling of host cells that affect inflammatory, immunological, and host-defence functions.\(^35\),\(^36\) Specific types of vaginal microbiota may sculpt the cervical cytokines in ways that influence the persistence of HPV infection or breach the incipient latency of the persistent hrHPV infection state and drive it towards induction of malignancy in the cervix.\(^35\)

Furthermore, health behaviours, including intravaginal health practice, number of sexual partners and other factors, may affect the types of vaginal microbiota and their association with risk of persistent hrHPV infection. There have been few studies of the vaginal microbiota\(^37\),\(^38\) and the interaction between the vaginal microbiota and cervical cytokines and their association with persistent hrHPV infection.\(^27\),\(^28\)

The design of the African Collaborative Center for Microbiome and Genomics Research (ACCME) HPV and Cervical Cancer Cohort Study enables us to study environmental, microbiomic, genetic and epigenetic factors associated with persistent hrHPV infection, in order to improve knowledge of the mechanism of HPV carcinogenesis and discover biomarkers of persistent hrHPV infection and cervical cancer. We evaluate the epidemiological determinants of persistent infection, and the genetic and epigenetic changes in hrHPV as well as in somatic cervical cells and their association with persistent hrHPV infection and CIN2+. We evaluate the epidemiological determinants of patterns of cervical cytokines and their association with persistent hrHPV infection. We identify the community state types and stability of the vaginal microbiota, and their association with persistent hrHPV infection. We conduct genome-wide association studies (GWAS) to identify the genetic variants associated with the risk of persistent hrHPV infection and CIN2+.

The ACCME cohort is located in Nigeria. Ethical approval to conduct this study was obtained from the National Health Research Ethics Committee in Nigeria. All study participants were informed about the study and were requested to consent before participation. The informed consent is reiterated at different study visits and new consent is obtained for specific components of the research project. An ethics and regulatory affairs coordinator conducts regular audits of the informed consent process and evaluates the understanding of the informed consent among randomly selected study participants. This study is funded by the National Institutes of Health.

Who is in the cohort?

There are 36 states, six geopolitical zones and a Federal Capital Territory in Nigeria. Our study is located in Abuja, the main municipality in the Federal Capital Territory, which is located in the centre of the country. In general, the socio-demographic characteristics of women in Abuja,
North Central Nigeria, were similar to those of women in
the South East, South South, and South West zones in
2013, but not the North East or North West which are
more rural and the women there are less likely to be edu-
cated or employed. 39 We randomly selected seven out of
42 districts in Abuja for our study. The populations served
by these study sites varied from urban city dwellers to
semi-rural to rural people living on farmlands and villages.
In each district, we employed extensive community engage-
ment strategies, to create awareness of the study and en-
sure that a representative sample of the target population
was enrolled. These strategies included: focus group dis-
cussions and surveys to identify cultural issues, literacy lev-
els and local language; town hall meetings and community
forums to gain input from the general public; participatory
evaluation; and partnerships with community stakeholders
to create alliances and ownership and build trust. We also
created awareness of the study through the use of: mass
media including radio and television talk shows; social
media including Twitter and Facebook; interactions with
key opinion leaders; and engagement of religious leaders,
women’s advocacy groups, corporate organizations, the
Nigerian Federal Ministry of Health and community
members.

In this study, we identified women who were at least 18
years old, had had sexual intercourse and had no previous
history of cervical abnormalities, cervical cancer or total
abdominal hysterectomy, by area sampling in Abuja.
Potential participants were offered HIV testing with volun-
tary counselling. Those who were HIV-positive were not
eligible to participate in the cohort and were referred to
free HIV treatment programmes. Enrolment into the co-
hort began in February 2014 in Abuja, Central Nigeria.

How often is follow-up?
Data are collected during the initial visit and at 6, 12, 18
and 24 months. Research nurses collect epidemiological
data using our tablet computer-assisted survey instruments
(TaCASI) directly into a secure web database applica-
tion—the Research Electronic Data Capture (REDCap)
platform hosted at the Institute of Human Virology
(IHVN). 43 Paper copies of the study forms are available to
serve as backup for data collection. Detailed contact infor-
mation (address and phone numbers) are collected from all
participants. Follow-up visits are scheduled at appropriate
times and reminders are sent by text messages, e-mails and
phone calls. Where participants cannot be reached by
phone, home visits are conducted.

What is being measured?
The data collection tools were piloted in a study of 1000
women with similar characteristics as the participants of
the ACCME study. In order to compute socioeconomic
status (SES) in a low-resource environment where in-
come data are sparse, we generated wealth index data as
previously described. 44 In summary, we used principal
components analysis (PCA) with varimax rotation to com-
pute factor scores based on the sum of the ownership of
household items weighted by their factor loading. We
sorted the data on the first principal component which had
the highest eigen value, and divided all respondents into
three categories based on its value. Participants with the
lowest 40% were categorized as low SES, the middle 40%
were categorized as middle SES and the top 20% were
categorized as high SES. The validity and reproducibility
of the wealth index has been examined in previous studies
and it correlates well with other measures of wealth in en-
vironments without reliable expenditure data. 44

We adapted tools for the measurement of alcohol in-
take, cigarette smoking, tobacco use, sexual and repro-
ductive health and medical and drug history from the
PhenX toolkit version of 20 September 2013, version
5.6. 45 We obtained self-report of occurrence of diseases
such as cancer, diabetes, myocardial infarction and stroke.
We modified the Harvard School of Public Health’s
Nurses’ Health Study II physical activity questionnaire
to collect data on physical activity and we used the Nigerian
food frequency questionnaire that we had previously de-
developed to collect information on dietary intake. Members
of the ACCME research group have used these tools for
previous research in Africa. 46–48 We asked participants
about their sexual activities in the past 24 h including his-
tory of sexual intercourse (vaginal, anal, oral), use of
contraceptives, sex toys and lubricants, and vaginal symp-
toms. Given the sensitive nature of some questions, espe-
cially those on sexual history, we ensured the mode and
placement of the questions were appropriate, the inter-
viewers were trained to be culturally and morally sensitive
and each interview was conducted in a relaxed, private set-
ing, sufficiently so to encourage accurate responses.

Three blood pressure (BP) measurements are taken at
least 1 min apart in accordance with the American Heart
Association recommendations. 49 using the automated
OMRON BP760 (HEM-7220-Z) with patients in a sitting
position, not earlier than 15 min after participant arrival
at the study site. Standing height, body weight and waist
and hip circumferences were measured in accord-
ance with the World Health Organization (WHO)
Multinational Monitoring of Trends in Cardiovascular
Disease (MONICA) project. 50 Pelvic examinations were
performed on all participants and data on any significant findings in the lower abdomen, the vulva/perineum, vagina, cervix and adnexa were collected as described.51,52 We performed bivale speculum examination and measured the vaginal pH using pH paper (pHydrion®, Micro Essentials Laboratories, Brooklyn, NY) and compared colour change of the pH paper with the manufacturer-provided colour charts.53,34 All research nurses passed a colour perception test before performing the pH tests. The data collected by questionnaires and during the Clinical Evaluation, Sample Evaluation and Testing schedule are outlined in Supplementary Table 1 (available as Supplementary data at IJE online).

Biological samples including blood and mid-vaginal and ectocervical cell samples are collected at baseline and during follow-up visits. The blood samples are separated into plasma, serum, buffy coat, red blood cells and clot. All biological samples are stored at -80°C at the ACCME Laboratories, IHVN, Abuja, Nigeria. Germline, somatic and viral DNAs are extracted using MagNa Pure LC 2.0® and Qiagen Qiacube HT robotic nucleic acid isolation and purification platforms. Germline DNA is quantitated using NanoDrop 8000 UV-Vis spectrophotometer at wavelengths of 260 and 280 nm, after which working dilutions to the 5–10 ng of DNA required per reaction are created.

Cervical exfoliated samples are analysed for any HPV and hrHPV using SPF10 PCR-DEIA-LiPA25, version 1, according to manufacturer’s instructions. Samples of cervical cytokines are collected and stored immediately in ice coolers and transported to the laboratory for storage. Cervical cytokines are measured using polystyrene non-magnetic bead-based multiplex assay according to manufacturer’s instructions (Bio-Rad® Bio-Plex 200 System®, Hercules, CA). The vaginal microbial species composition and abundance will be determined as described by Forney et al.10 The V1-V3 hypervariable regions of the 16S rRNA genes will be amplified using an optimized primer set 27F and 533R as recommended by the Human Microbiome Project (HMP) [http://www.hmpdacc.org]. Colposcopy and biopsy will be done for all individuals with persistent hrHPV infection and clinical features of a cervical lesion suspected to be CIN2+ and for matching controls. Biopsy samples are handled according to the TCGA [http://cancergenome.nih.gov] standard operating procedure. Spot urine samples are collected and tested for glucose, ketones, specific gravity, blood, pH, protein, nitrites and leukocytes with Multistix® 10 SG reagent strip; the rest are stored in the laboratory at -80°C. Women with cervical cancer are biopsied and referred for appropriate treatment. We store fresh frozen samples and paraffin embedded samples of the cervical biopsy and collect minimal data from these women; they are not enrolled in the prospective cohort.

Quality assurance and control

Data
Tablet computer-assisted survey instruments (TaCASI) have proved versatile in survey research that incorporates sensitive questions like those on sexual behaviour.55–57 We use real-time data entry into secure RedCap databases with in-built logic and error checks, which enables data managers to review data and follow-up on missing values and outliers with site research associates promptly.11,12,53–59

Laboratory
Two independent pathologists who are blinded to the HPV status of the participants report on the histological diagnoses. Quality assurance (QA) and quality control (QC) of HPV genotyping is done in collaboration with DDL diagnostic laboratory. Human genomics QA/QC is done in collaboration with the Center for Research on Genomics and Global Health, at the National Human Genome Research Institute. We store digital colposcopy images on a private cloud server for secondary review.

What has been found?
The focus of this project so far has been on establishing the study population and obtaining baseline data on known and potential risk factors of hrHPV infection and CIN2+, which will be used for epidemiological and genomic studies. Some early results are presented here.

From commencement of enrolment in February 2014 to July 2016, 11 500 women had been enrolled in the ACCME cohort. The women in this cohort have homogeneous contraceptive, sexual and reproductive characteristics and health status compared with similarly aged HIV-negative women, in the general population.40–42 Many of the women enrolled were in their third or fourth decade of life, the mean age [standard deviation (SD)] of the participants was 39 (10) years. Most of the participants were married (77%; 8832/11 500), monogamous and live with their spouses (86%; 7556/8832). Many participants have had some university education (45%; 5152/11 500) and have professional jobs (36%; 4175/11 500). Selected socio-demographic characteristics of the study participants at baseline are shown in Table 1. To date we have tested the baseline samples of all the study participants for HPV using DEIA, and observed that 42% (4773/11 500) tested positive (Table 2). Similar tests for HPV have been done in 5349 participants at the 12 months follow-up visit, and we observed that 21% (1107/5349) of these women had persistent HPV infections. About 16% of the study participants have had oral sex and < 1% have had anal sex.
Table 3. Most participants thought anal sex was unacceptable for health (57%), religious (53%) and/or cultural (26%) reasons (multiple responses allowed). Some of the participants reported a history of physician-diagnosed hypertension (15%), diabetes (2%), hypercholesterolaemia (4%) or heart disease (0.3%) (Table 4). We found the mean (SD) vaginal pH was 5.2 (0.5); it was similar among HPV-positive and HPV-negative women. Selected baseline socio-demographic characteristics of women in the ACCME Cohort, n (%)
Table 2. Prevalence (%) of HPV infection among women in the ACCME cohort

| HPV status                             | Any HPV<sup>a</sup> | Low-risk HPV<sup>b</sup> | High-risk HPV<sup>c</sup> |
|----------------------------------------|----------------------|-------------------------|--------------------------|
| HPV-positive at baseline               | 4773 (41.5)          | 1718 (36.0)             | 3050 (63.9)              |
| HPV-positive at follow-up visit<sup>c</sup> | 2305 (43.1)          | 521 (22.6)              | 1784 (77.4)              |
| Persistent HPV-positive                | 1107 (20.7)          | 139 (12.6)              | 598 (54.0)               |

<sup>a</sup>Based on DEIA.
<sup>b</sup>Results based on LiPA.
<sup>c</sup>Follow-up visit at 12 months after baseline, current total n = 5349.

Table 3. Some baseline sexual and reproductive history of women in the ACCME cohort

| Characteristics                             | Total (n = 11500) | HPV-negative (n = 6727) | HPV-positive (n = 4773) |
|--------------------------------------------|-------------------|------------------------|------------------------|
| Mean (standard deviation)                  |                   |                        |                        |
| Age at menarche                            | 14 (2)            | 14 (2)                 | 14 (2)                 |
| Age at menopause                           | 44 (9)            | 44 (9)                 | 43 (9)                 |
| Age at first vaginal sexual intercourse     | 20 (4)            | 21 (4)                 | 20 (4)                 |
| Age at first oral sexual intercourse        | 26 (6)            | 27 (6)                 | 26 (6)                 |
| Age at first anal sexual intercourse        | 27 (7)            | 27 (7)                 | 26 (7)                 |
| Number of lifetime sexual partners         |                   |                        |                        |
| Vaginal                                    | 2.6 (2.6)         | 2.7 (2.6)              | 2.8 (2.7)              |
| Oral                                       | 1.8 (1.7)         | 1.6 (1.5)              | 1.8 (1.8)              |
| Anal                                       | 1.3 (1.2)         | 1.0 (0.6)              | 1.3 (1.2)              |
| Types of sexual experience<sup>d</sup>     |                   |                        |                        |
| Vaginal                                    | 11500 (100.0)     | 6727 (100.0)           | 4773 (100.0)           |
| Oral                                       | 1832 (15.9)       | 1016 (15.1)            | 816 (17.1)             |
| Anal                                       | 69 (0.6)          | 42 (0.6)               | 27 (0.6)               |
| First type of sexual experience            |                   |                        |                        |
| Vaginal                                    | 9574 (98.3)       | 5605 (98.4)            | 3969 (98.1)            |
| Oral                                       | 154 (1.6)         | 8 (1.5)                | 3 (1.8)                |
| Anal                                       | 11 (0.1)          | 82 (0.1)               | 72 (0.1)               |
| Oral sex type<sup>e</sup>                  |                   |                        |                        |
| Fellatio                                   | 964 (58.2)        | 545 (59.3)             | 419 (56.8)             |
| Cunnilingus                                | 689 (41.6)        | 371 (40.4)             | 318 (43.1)             |
| Anallingus                                 | 4 (0.2)           | 3 (0.3)                | 1 (0.1)                |
| Anal sex type<sup>e</sup>                  |                   |                        |                        |
| Receptive                                  | 195 (94.4)        | 109 (55.9)             | 86 (44.1)              |
| Insertive (using objects e.g. sex toys)    | 1 (0.5)           | 0 (0.0)                | 1 (100.0)              |
| Both                                       | 9 (5.1)           | 6 (66.7)               | 3 (33.3)               |
| Sexual type preferred                      |                   |                        |                        |
| None                                       | 75 (0.8)          | 52 (0.9)               | 23 (0.6)               |
| Vaginal                                    | 9363 (97.3)       | 5484 (97.2)            | 3879 (96.9)            |
| Oral                                       | 14 (2.0)          | 9 (1.7)                | 5 (2.4)                |
| Anal                                       | 196 (0.2)         | 99 (0.2)               | 97 (0.1)               |
| Usual frequency of sexual intercourse      |                   |                        |                        |
| Vaginal                                    |                   |                        |                        |
| < 1/month                                  | 3186 (32.5)       | 1826 (32.0)            | 1342 (33.2)            |
| 1–3/month                                  | 1825 (18.7)       | 1010 (17.7)            | 815 (20.2)             |
| 1/week                                     | 2339 (24.0)       | 1410 (24.7)            | 929 (23.0)             |
| 2–4/week                                   | 2075 (21.3)       | 1254 (22.0)            | 821 (20.3)             |
| ≥ 5/week                                   | 335 (3.5)         | 202 (3.6)              | 133 (3.3)              |

(Continued)
Table 3. Continued

| Characteristics                        | Total     | HPV-negative | HPV-positive |
|----------------------------------------|-----------|--------------|--------------|
|                                        | \( n = 11500 \) | \( n = 6727 \) | \( n = 4773 \) |
| **Oral**                               |           |--------------|--------------|
| < 1/month                              | 9208 (95.1) | 5392 (95.0) | 3816 (95.1) |
| 1–3/month                              | 233 (2.4)  | 125 (2.2)   | 108 (2.7)   |
| 1/week                                 | 101 (1.0)  | 65 (1.2)    | 36 (0.9)    |
| 2–4/week                               | 105 (1.1)  | 65 (1.1)    | 40 (1.0)    |
| ≥ 5/week                               | 41 (0.4)   | 27 (0.5)    | 14 (0.3)    |
| **Anal**                               |           |--------------|--------------|
| < 1/month                              | 9635 (99.6) | 5643 (99.6) | 3992 (99.6) |
| 1–3/month                              | 25 (0.2)   | 12 (0.2)    | 13 (0.3)    |
| 1/week                                 | 6 (0.05)   | 3 (0.05)    | 3 (0.06)    |
| 2–4/week                               | 8 (0.07)   | 7 (0.1)     | 1 (0.02)    |
| ≥ 5/week                               | 4 (0.03)   | 3 (0.05)    | 1 (0.02)    |
| **Usual gender of sexual partners**    |           |--------------|--------------|
| Only males, never females              | 9546 (99.4) | 5578 (99.3) | 3968 (99.2) |
| Mostly males, rarely females           | 54 (0.5)   | 28 (0.6)    | 24 (0.7)    |
| Only females, never males              | 10 (0.1)   | 5 (0.1)     | 5 (0.1)     |
| Mostly females, rarely males           | 7 (1.0)    | 6 (0.1)     | 1 (0.1)     |
| **Menopausal status**                  |           |--------------|--------------|
| Premenopausal                          | 8049 (82.7) | 4704 (82.7) | 3345 (82.8) |
| Postmenopausal                         | 1678 (17.3) | 985 (17.3)  | 693 (17.2)  |
| **Ever been pregnant**                 |           |--------------|--------------|
| Yes                                    | 8698 (89.1) | 5163 (90.4) | 3535 (87.3) |
| No                                     | 1061 (10.9) | 545 (9.6)   | 516 (12.7)  |

*Multiple responses allowed. HPV results are based on DEIA tests at baseline only. Given the sensitive nature of the sexual and reproducibility history questions, some women did not respond. Therefore the total does not sum up to 11 500.

Table 4. Baseline history of selected physician-diagnosed medical conditions among women in the ACCME cohort, \( n \) (%)

| Disease                  | Mean age at diagnosis (years) | Total (%) | Rural (%) | Semi-rural (%) | Urban (%) |
|--------------------------|-------------------------------|-----------|-----------|----------------|-----------|
|                          | \( n = 11500 \)               | \( n = 1380 \) | \( n = 5336 \) | \( n = 4784 \) |
| **Hypertension**         |                               |           |           |                |           |
| Yes                      | 40 (8)                        | 1702 (14.8) | 187 (13.5) | 630 (11.8)    | 885 (18.5) |
| No                       | –                             | 9798 (85.2) | 1193 (86.5) | 4706 (88.2)  | 3899 (81.5) |
| **Diabetes**             |                               |           |           |                |           |
| Yes                      | 43 (8)                        | 219 (1.9)  | 21 (1.5)  | 69 (1.3)      | 129 (2.7)  |
| No                       | –                             | 11281 (98.1) | 1359 (98.5) | 5267 (98.7)  | 4653 (97.3) |
| **Hypercholesterolaemia**|                               |           |           |                |           |
| Yes                      | 43 (8)                        | 552 (4.1)  | 108 (7.8) | 133 (2.5)     | 311 (6.5)  |
| No                       | –                             | 10948 (95.2) | 1272 (92.2) | 5203 (97.5)  | 4473 (93.5) |
| **Rheumatic fever**      |                               |           |           |                |           |
| Yes                      | 39 (10)                       | 58 (0.5)  | 23 (1.7)  | 21 (0.4)      | 14 (0.3)   |
| No                       | –                             | 11442 (99.5) | 1357 (98.3) | 5315 (99.6)  | 4770 (99.7) |
| **Heart disease**        |                               |           |           |                |           |
| Yes                      | 40 (10)                       | 35 (0.3)  | 0 (0.0)   | 11 (0.2)      | 24 (0.5)   |
| No                       | –                             | 11465 (99.7) | 1380 (99.8) | 5325 (99.8)  | 4760 (99.5) |
| **TIA/stroke**           |                               |           |           |                |           |
| Yes                      | 42 (9)                        | 35 (0.3)  | 5 (0.4)   | 16 (0.3)      | 14 (0.3)   |
| No                       | –                             | 11465 (99.7) | 1375 (99.6) | 5320 (99.7)  | 4770 (99.7) |
| **Kidney disease**       |                               |           |           |                |           |
| Yes                      | 45 (9)                        | 46 (0.4)  | 6 (0.4)   | 11 (0.2)      | 29 (0.6)   |
| No                       | –                             | 11454 (99.6) | 1374 (99.6) | 5325 (99.8)  | 4755 (99.4) |
| **Cancer**               |                               |           |           |                |           |
| Yes                      | 41 (10)                       | 23 (0.2)  | 7 (0.5)   | 6 (1.1)       | 10 (1.2)   |
| No                       | –                             | 11477 (99.8) | 1373 (99.5) | 5330 (99.9)  | 4774 (99.8) |
Table 5. Selected baseline gynaecological characteristics of women in the ACCME Cohort, n (%)

| Gynaecological characteristics | Total n = 11500 | HPV-negative n = 6727 | HPV-positive n = 4773 |
|-------------------------------|----------------|-----------------------|----------------------|
| Vaginal pH<sup>a</sup>        | 5.2 (0.5)      | 5.2 (0.5)             | 5.2 (0.6)            |
| Ectopy observed               |                |                       |                      |
| Yes                           | 1368 (11.9)    | 809 (12.0)            | 559 (11.7)           |
| No                            | 10132 (88.1)   | 5918 (88.0)           | 4214 (88.3)          |
| Transformation zone           |                |                       |                      |
| < 25%                         | 3324 (28.9)    | 1978 (29.4)           | 1346 (28.2)          |
| 25–50%                        | 4310 (37.5)    | 2382 (35.4)           | 1928 (40.4)          |
| 51–75%                        | 2901 (25.2)    | 1823 (27.1)           | 1078 (22.6)          |
| > 75%                         | 965 (8.4)      | 544 (8.1)             | 421 (8.8)            |
| Squamo-columnar junction      |                |                       |                      |
| Fully observed                | 8245 (71.7)    | 4877 (72.5)           | 3368 (71.1)          |
| Partially observed            | 2369 (20.6)    | 1406 (20.9)           | 963 (20.1)           |
| Not observed                  | 886 (7.7)      | 444 (6.6)             | 422 (8.8)            |
| Cervical friability<sup>b</sup>|                |                       |                      |
| None                          | 10568 (91.9)   | 6168 (91.7)           | 4400 (92.2)          |
| Mild                          | 839 (7.3)      | 511 (7.6)             | 328 (6.9)            |
| Moderate                      | 93 (0.8)       | 48 (0.7)              | 45 (0.9)             |

HPV status based on DNA enzyme immunoassay (DEIA) test.
<sup>a</sup>Mean (standard deviation).
<sup>b</sup>Cervical friability: mild, discrete spot of blood on swab; moderate, pink discolouration, swab soaked.

gynaecological characteristics of the study participants at baseline are shown in Table 5. Our results on sexual health and behaviour provide important data for studies of associations between these characteristics and HPV-associated cancers including cervical, head and neck, and anal cancers, and identifies attitudes and beliefs which may contribute to the social epidemiological risk for other non-communicable diseases (NCD). The study attrition rate is ~ 20%.

Training and capacity development

The ACCME project includes a training programme in epidemiology, molecular biology, data management and analysis. Several pre- and postdoctoral trainees are currently engaged in the project and are taking the lead in several analyses and publications. Students and faculty members from Nigerian and US universities and research institutes have also used the resources of the project for their own research projects.

Future plans?

We will contribute DNA samples from our study participants to the H3Africa Biorepositories according to the H3Africa guidelines. We are participating in the development of the H3Africa Consortium Genome Analysis Array chip in collaboration with other H3Africa projects and Illumina Inc. The resultant chip will be highly informative for genomics research in African populations. We plan to replicate our genomics findings in other African cohorts. The combination of somatic and germline mutation analyses in our study enhances opportunities for gene discovery, understanding of gene functions, new clinical insights and integrative analysis of cervical cancer. Our research will also characterize the epidemiology of HPV infection in Nigeria before widespread deployment of HPV vaccination. We intend to maintain and expand this valuable cohort to include male partners of the participants and to study other NCDs in future.

What are the main strengths and weaknesses?

The ACCME cohort incorporates a large number of repeated measurements of a wide range of exposures. There are also strong data and laboratory QA/QC procedures in collaboration with local and international researchers incorporated into the project, thereby ensuring high quality of the data. An ethics and regulatory compliance officer independently monitors research activities at clinical sites and generates reports for the Study leadership for action. Close collaboration with the NIH-funded West African Bioethics Training Program [http://bioethicscenter.net] ensures ongoing training in research ethics, good clinical and laboratory practices, and responsible conduct of research. We implement extensive community engagement efforts that include: community rallies; regular meetings with research participants; circulation of study newsletters that provide updates about study...
progress and challenges; and motivational and health education messages through e-mails, radio, TV and newspapers. The study maintains a webpage, a Facebook page and an active Twitter account. Study participants are able to contact the research staff and leadership via phone applications, including Blackberry messenger and WhatsApp.

A major limitation of longitudinal studies is loss to follow-up. This can be particularly challenging in low-resource environments where participants have poor history of follow-up even in clinical care. To improve participant retention in the cohort, we deployed several strategies including extensive use of mobile health-based interventions such as automated phone applications that are used to send health tips and visit reminders to participants before their scheduled appointment, regularly. Thus, we have achieved a participant retention rate of ~80%.

Problems associated with conducting research in resource-limited settings that may affect ACCME include poor infrastructure, inadequate power supply, challenges with conduct of research in low-literacy environments and lack of trained personnel. To address these, we: purchased state of the art laboratory equipment with service agreements for genomic analyses; set up a three-level power backup system with multiple power generators, inverters and batteries for the laboratory; develop appropriate health education materials; train and re-train all research staff; and implement schemes for motivation of staff through regular research meetings and opportunities to attend international meetings, implementation of mentored research projects and generation of appropriate health education materials. All of these challenges have contributed to much higher cost for implementation of this research than anticipated. Nonetheless, we collaborate with renowned international institutions in the USA, UK and The Netherlands for ongoing staff training and support.

Can I get hold of the data? Where can I find out more?

Further information is available at [http://h3africa.org/]. Documentation for the ACCME cohort including the questionnaires, information sent to the participants and detailed information about the research, is available at [http://h3acme.com/]. We also welcome specific queries and proposals for collaboration, which should be directed to the scientific director [sadebamowo@som.umaryland.edu] and the principal investigator [cadebamowo@som.umaryland.edu] of ACCME.

Supplementary Data

Supplementary data are available at IJE online.

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References

1. IARC. Globocon 2012:Estimated Incidence, Mortality and Prevalence Worldwide in 2012. 2012. [http://globocan.iarc.fr/Pages/ fact_sheets_cancer.aspx (November 2014, date last accessed).]
2. Jedy-Agba E, Oga E, Oduotula M et al. Cancer incidence in Nigeria from 2009 to 2013. Ann Glob Health 2015;81:92.
3. Ferlay J, Soerjomataram I, Ervik M et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. 2013. [http://globocan.iarc.fr/Pages/ fact_sheets_cancer.aspx (November 2014, date last accessed).]
4. Forman D, de Martel C, Lacey CJ et al. The epidemiology of genital human papillomavirus infection in a cohort of closely followed adolescent women. Vaccine 2012;30(Suppl 5):F12–23.
5. Stanley M. Immunochemistry of HPV and HPV vaccines. Gynecol Oncol 2008;109(Suppl 2):S15–21.
6. Brown DR, Shew ML, Qadadi B et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. Infect Dis 2005;191:182–92.
7. Trotter H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006;24(Suppl 1):S1–15.
8. McCredie MRE, Sharples KJ, Paul C et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. Lancet Oncol 2008;9:425–34.
9. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2:342–50.
10. Dartell M, Rasch V, Kaheisa C et al. Human papillomavirus prevalence and type distribution in 3603 HIV-positive and HIV-
negative women in the general population of Tanzania: the PROTECT study. Sex Transm Dis 2012;39:201–08.
11. Akarolo-Anthony SN, Al-Mujtaba M, Famooto AO et al. HIV associated high-risk HPV infection among Nigerian women. BMC Infect Dis 2013;13:521.
12. Akarolo-Anthony SN, Famooto AO, Dareng EO et al. Age-specific prevalence of human papilloma virus infection among Nigerian women. BMC Public Health 2014;14:656.
13. Dalal S, Beunza JJ, Volmink J et al. Non-communicable diseases in sub-Saharan Africa: what we know now. Int J Epidemiol 2011;40:885–901.
14. Holmes MD, Dalal S, Volmink J et al. Non-communicable diseases in sub-Saharan Africa. Part II - The case for cohort studies. PLoS Med 2010;7:e1000244.
15. Smith JS, Lindsay L, Hoots B et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121:621–32.
16. Walboomers JM, Jacobs MV, Manos MM et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12–19.
17. Schmeink CE, Melchers WJ, Siebers AG, Quint WG, Massuger LF, Bekkers RL. Human papillomavirus persistence in young unscreened women, a prospective cohort study. PLoS One 2011;6:e27937.
18. Li N, Franceschi S, Howell-Jones R, Snijders PJF, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer 2011;128:927–35.
19. de Freitas AC, Gurgel AP, Chagas BS, Coimbra EC, do Amaral CM. Susceptibility to cervical cancer: an overview. Gynecol Oncol 2012;126:304–11.
20. Anorlu R. Cervical cancer: the sub-Saharan African perspective. Reprod Health Matters 2008;16:41–49.
21. Denny L, Anorlu R. Cervical cancer in Africa. Cancer Epidemiol Biomarkers Prev 2012;21:1434–38.
22. Ahlbom A, Lichtenstein P, Malmstrom H, Fyechting M, Hemminki K, Pedersen NL. Cancer in twinning-genetic and non-genetic familial risk factors. J Natl Cancer Inst 1997;89:287–93.
23. Chattopadhyay K. A comprehensive review on host genetic susceptibility to human papillomavirus infection and progression to cervical cancer. Indian J Hum Genet 2011;17:132–44.
24. Zelmanowicz A, Hildesheim A. Family history of cancer as a risk factor for cervical carcinoma: A review of the literature. Papilloma Virus Rep 2004;15:113–20.
25. Moore EE, Wark JD, Hopper JL, Erbas B, Garland SM; CeCaGeEn Study Group. The roles of genetic and environmental factors on risk of cervical cancer: a review of classical twin studies. Twin Res Hum Genet 2012;15:79–86.
26. Magnuson PK, Lichtenstein P, Glynnsten UB. Heritability of cervical tumours. Int J Cancer 2000;88(5):698–701.
27. Hemminki K, Dong C, Vaittinen P. Familial risks in cervical cancer: is there a hereditary component? Int J Cancer 1999;82:775–81.
28. Benschop CC, Quaak FC, Boon ME, Sijen T, Kuiper I. Vaginal microbiial flora analysis by next generation sequencing and microarrays: can microbes indicate vaginal origin in a forensic contest? Int J Legal Med 2012;126:303–10.
29. Fettweis JM, Serrano MG, Girerd PH, Jefferson KK, Buck GA. A new era of the vaginal microbiome: advances using next-generation sequencing. Chem Biodiversity 2012;9:965–76.
30. Forney LJ, Gajer P, Williams CJ et al. Comparison of self-collected and physician-collected vaginal swabs for microbiome analysis. J Clin Microbiol 2010;48:1741–48.
31. Gloo GR, Hummelen R, Macklaim JM et al. Microbiome profiling by Illumina sequencing of combinatorial sequence-tagged PCR products. PLoS One 2010;5:e15406.
32. Hyman RW, Herndon CN, Jiang H et al. The dynamics of the vaginal microbiome during infertility therapy with in vitro fertilization-embryo transfer. J Assist Reprod Genet 2012;29:105–15.
33. Ma B, Forney LJ, Ravel J. Vaginal Microbiome: Rethinking Health and Disease. Ann Rev Microbiol 2012;66:371–89.
34. Ravel J, Gajer P, Abdo Z et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A 2011;108(Suppl 1):4680–87.
35. Barton ES, White DW, Cathelyn JS et al. Herpesvirus latency confers symbiotic protection from bacterial infection. Nature 2007;447:326–29.
36. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature 2007;449:811–18.
37. Schellenberg JJ, Links MG, Hill JE et al. Molecular definition of vaginal microbiota in East African commercial sex workers. Appl Environ Microbiol 2011;77:4066–74.
38. Hummelen R, Fernandes AD, Macklaim JM et al. Deep sequencing of the vaginal microbiota of women with HIV. PLoS One 2010;5:e12078.
39. National Population Commission Nigeria Demographic and Health Survey. Abuja: NPC, 2013.
40. Akinsoji AA, Olufunmilola AA, Idowu AA, Pius AO. Sexual and Contraceptive Practices among Female Undergraduates in a Nigerian Tertiary Institution. Ethiop J Health Sci 2015;25:209–16.
41. Yaksai IA, Ugwa EA, Orubu J. Gynecological malignancies in Aminu Kano Teaching Hospital Kano: a 3 year review. Niger J Clin Pract 2013;16:63–66.
42. Umeora O, Egwuatu V. Age at menarche and the menstrual pattern of Igbo women of southeast Nigeria. Afr J Reprod Health 2008;12:90–95.
43. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap) - a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42:377–81.
44. Filmer D, Pritchett LH. Estimating wealth effects without expenditure data - or tears: an application to educational enrollments in states of India. Demography 2001;38:115–32.
45. Hamilton CM, Strader LC, Pratt JG et al. The PhenX Toolkit: get your most from your measures. Am J Epidemiol 2011;174:253–60.
46. Akarolo-Anthony SN, Oduobre FO, Yilme S et al. Pattern of dietary carbohydrate intake among urbanized adult Nigerians. Int J Food Sci Nutr 2013;64:292–99.
47. Akarolo-Anthony SN, Adebamowo CA. Prevalence and correlates of leisure-time physical activity among Nigerians. BMC Public Health 2014;14:529.
48. Akarolo-Anthony SN, Willett WC, Spiegelman D, Adebamowo CA. Obesity epidemic has emerged among Nigerians. *BMC Public Health* 2014;14:455.

49. Pickering TG, Hall JE, Appel LJ et al. Recommendations for blood pressure measurement in humans: an AHA scientific statement from the Council on High Blood Pressure Research Professional and Public Education Subcommittee. *J Clin Hypertens (Greenwich)* 2005;7:102–09.

50. WHO. MONICA Manual Part III: Population Survey. Section 1: Population Survey Data Component. 1997. http://www.thl.fi/publications/monica/manual/part3/iii-1.htm (30 September 2014, date last accessed).

51. Fergusson CM. Inspection, auscultation, palpation, and percussion of the abdomen. In: Walker HK HW, Hurst JW (eds). *Clinical Methods: The History, Physical and Laboratory Examinations*. 3rd edn. Boston, MA: Butterworths, 1990.

52. Long WN. Pelvic examination. In: Walker HK HW, Hurst JW, (eds). *Clinical Methods: The History, Physical and Laboratory Examinations*. Boston, MA: Butterworths, 1990.

53. Heinze T, Riedewald S, Saling E. Determination of vaginal pH by pH indicator strip and by pH micro electrode. *J Perinat Med* 1989;17:477–79.

54. Huppert JS, Bates JR, Weber AF, Quinn N, Gaydos CA. Abnormal vaginal pH and Mycoplasma genitalium infection. *J Pediatr Adolesc Gynecol* 2013;26:36–39.

55. Angad M, Alfiaa AS. Integrating Web 2.0 in Clinical Research Education in a Developing Country. *J Cancer Educ* 2014;29:536–40.

56. Franklin JD, Guidry A, Brinkley JF. A partnership approach for Electronic Data Capture in small-scale clinical trials. *J Biomed Inform* 2011;44(Suppl 1):S103–08.

57. Dupont A, Wheeler J, Herndon JE 2nd, et al. Use of tablet personal computers for sensitive patient-reported information. *J Support Oncol* 2009;7(3):91–7.

58. Dareng EO, Ma B, Famooto AO et al. Prevalent high-risk HPV infection and vaginal microbiota in Nigerian women. *Epidemiol Infect* 2016;144:123–37.

59. Famooto A, Almujtaba M, Dareng E et al. RPS19 and TYMS SNPs and Prevalent High Risk Human Papilloma Virus Infection in Nigerian Women. *PloS One* 2013;8:e66930.