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Molecular characterization of lower vaginal swabs for Human papilloma virus in association with *Chlamydia trachomatis* infection in Cameroonian Women

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ABSTRACT

Human papilloma virus (HPV) infection is an etiological factor for cervical cancer development and *Chlamydia trachomatis* (Ct) is considered as a cofactor. Understanding the dynamics of HPV and Ct infection could help to explain the incidence of early onset of cervical cancer (CC) observed in Cameroon.

Lower vaginal swabs and sera from sexually active women were analyzed for HPV and Ct infection in association with risk factors. Questionnaires were used to document patients' lifestyle and risk factors.

A total of 206 women participated in the study average 28.1 ± 8 years (16–50 years). HPV prevalence was 23.3% with subtypes 16 and 18 at respectively 2.9% and 1%. Ct infection totalised 40.8%, of which 23.8% were HPV- Ct co-infections. HPV infection was inversely associated with age ($p = 0.028$). We found a positive association between Ct infection and the number of sex partners ($p = 0.012$) and a negative association with parity ($p = 0.032$). There was no significant association between HPV and Ct infections.

High rates of HPV and Ct infections could be an indicator of cervical cancer risk in the near future. There is therefore an urgent need for sensitization as well as implementation of appropriate preventive measures.

Introduction

Human papilloma viruses (HPV) infections are among the most prevalent sexually transmitted infections in men and women in developing countries [1]. Meanwhile several HPV subtypes are known, a subset is highly associated with risk of cervical cancer development [2,3]. Recent estimates indicate that worldwide burden of HPV infections stands between 11% and 12% with large geographical differences. The highest incidence rates are observed in sub-Saharan Africa (24%), Eastern Europe (21%) and Latin America (16%) [4]. Of all HPV subtypes, HPV 16 and 18 are the most prevalent high risk subtypes with global incidence rates of 3.2% and 1.4% respectively [5]. The carcinogenicity of HPV has been definitively established for six human cancers (cervical, penis, vulva, vagina, anus and oropharynx), and of the 12.7 million human cancers reported in 2012, some 610,000 cases could clearly be attributed to HPV infection [4]. The numbers differ for different geographical regions, and are directly related to development index. For example, the HPV-attributable fraction of human cancers in sub-Saharan Africa is estimated to be around 14.2%, 15.4% in India and about 1.6% in North America [4]. Considering the lack of adequate and systematic HPV screening and vaccination programs in most less developed regions, the numbers could be even higher. HPV is therefore a clear public health problem in most developing regions and despite the lack of reliable epidemiological data, its health impact is by no means minimised.

Chlamydia trachomatis (Ct) on the other hand, is a major co-factor for HPV-driven cervical cancer and is known to be responsible for other diseases such as chronic inflammatory diseases [6]. According to recent reports, the global burden of CT infection is about 4.2% in women and 2.7% in men, representing some 130 million annual new cases [7]. Of the world Ct burden, more than two thirds occur in developing countries, with above 80%

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of cases remaining asymptomatic. Based on available data, incidence may go from 8% to more than 37% in some instances [8]. Besides rare and sporadic *Chlamydia* testing implemented in some countries, such high prevalence rates of asymptomatic infection lay firm grounds for proper transmission and does in no way minimise the role of Ct as a risk factor for HPV and HIV infections [9,10]. As a result of conflicting reports, the role of Ct infection in the development of HPV-driven cervical cancer still needs to clearly be elucidated. Some studies have reported independent association of Ct with squamous cell carcinoma [11] while others report no significant association [12]. Nonetheless, Ct implication in cancer development is much more established. Moreover, apart from its potential implication in cervical cancer development, Ct has been shown to have several reproductive health implications such as neonatal morbidity, pelvic inflammatory disease, infertility and ectopic pregnancies as well as other genital cancers [13]. HPV and Ct coinfections would therefore form a perfect lethal cocktail with unprecedented potentials of driving cervical cancer and other health-related problems. It is therefore evident, that such coinfections represent major public health issues, especially in low income settings thereby increasing the risk of cervical cancer development.

The global burden of cancer is being shifted to less developed countries [14] with about 57% of cancer cases registered in these regions and 65% of cancer-related deaths. Cervical cancer is the second most diagnosed and third leading cause of female cancer-related deaths in developing countries. The highest incidence rates occur in sub-Saharan Africa, Latin America, and Melanesia accounting for 90% of the World's cervical cancer deaths [14]. The geographical disparities in CC incidence could be explained by differences in the implementation of screening programs for early detection of precancerous lesions and HPV infections. In countries where systematic screening programs have been implemented, CC incidence has reduced by up to 65% as it is the case in Norway [14]. CC is one of the most prevalent female cancers world-wide and representing about 23% of all preventable cancer-related deaths in low and middle income countries [15]. Despite the fact that CC can be prevented by vaccination, no systematic vaccination programs have been implemented in Cameroon, as it is the case in many developing countries worldwide. In this context, cervical cancer prevention through behavior change remains the only alternative. Pushing for more investigation on infection risk factors in the Cameroonian population would provide alternative measures to reduce disease burden.

The present study evaluates the prevalence of HPV and Ct infections among sexually active women attending the Dschang district hospital, in Cameroon. The study evaluates the risk of infection-related cervical cancer and report the incidence rate of *Chlamydia* and HPV infections among sexually active women in the population.

Study site and population

The study was performed in Dschang, the divisional headquarter of the Menoua division of the west region of Cameroon. Dschang is a University town with several colleges and higher education institutes that cohabit in the city, making it an attractive commercial centre and constituting a very dynamic and active population. With a total area of 450 km^2 it has a total population of about 102,000 inhabitants involved in different works of life. Most inhabitants can be categorized under one of the following professional categories: house wives, students, traders and employees of the public sector. The transmission of sexually transmitted infections (STIs) could be high within such a population. Participants were randomly recruited during visits to the hospital for routine gynaecological check-ups. All participants consented to participate in the study by signing an informed consent form. Ethical clearance was obtained from the Dschang district hospital review board and the Cameroon Bioethics Initiative (CAMBIN).

Methods

Sampling

Participants were recruited between June 2015 and April 2016. Prior to that, the goal of the study was clearly explained to participants, and when consent was given, subjects were included into the study. A detailed questionnaire was administered to all participants to document their lifestyle patterns. All participants who could not read or write were assisted by the medical personnel, and the questions in some cases were well explained in the local languages. Low vaginal swabs were collected from participants for the molecular detection of HPV and the blood samples were used for the detection of *Chlamydia* infection by enzyme-linked immunosorbent assay (ELISA). Biological material from all swabs were immediately collected into a tube containing 1 ml of sterile saline (0.9% NaCl) and kept frozen at −20 °C until they were used for DNA extraction. Serum samples were prepared from the blood and aliquoted before being stored at −20 °C. The swab samples were transferred from the biochemistry laboratory of the Dschang district Hospital, to the Molecular parasitology and entomology Unit at the University of Dschang for molecular analyses. *Chlamydia* infection test was performed at the district hospital

Primer sequences used in the study.

Primer name	Sequence 5′ → 3′	Tm (°C)	Amplicon size (bp)
My09	cgtccmarrggawactgatc	52.5	450
My11	gcmcagggwcataayaatgg	55.8	
HPV16F	ttaggcagcacttggccaacca	62	207
HPV16R	taatccgtcctttgtgtgagct	67	
HPV18F	tcgcgtcctttatcacagggcga	63	274
HPV18R	tgcccaggtacaggagactgtg	66	

DNA extraction

For molecular diagnosis of HPV infections, DNA was extracted following the hexadecyltrimethylammonium bromide (CTAB) based method previously described [16] with some modifications. Briefly, frozen samples were thawed and 200 μl were pipetted into a new Eppendorf tube. The samples were centrifuged at 10,000 rpm on a benchtop centrifuge. The resulting pellet was washed twice in 500 μl of deionized water and re-suspended in 500 μl of CTAB buffer (CTAB 2%; 0.1 M Tris, pH 8; 0.02 M EDTA pH 8; 1.4 M NaCl). Samples were then incubated at 60 °C for 30 min for complete lysis and DNA was isolated from the lysate by means of a mixture of chloroform/isoamyl alcohol (24/1; V/V) extraction followed by precipitation with sodium acetate in isopropanol (V/V). After centrifugation at 13000 rpm for 15 min, the pellet was rinsed with 70% ethanol, air-dried, and re-suspended in 25 μl distilled sterile water. The DNA samples were diluted 25 folds and stored at −20 °C until PCR amplification.

Detection of HPV by PCR

Molecular identification of HPV was achieved by polymerase chain reaction (PCR) using the MY09/MY11 consensus L1 primers for all HPV subtypes [17] with some modifications. Briefly, a PCR master mix comprising of 1× Q5 high fidelity™ reaction buffer (New England Biolabs), 0.4 μM of each primer, 400 μM dNTPs and 0.5U of Q5 high fidelity DNA polymerase were prepared and distributed into PCR tubes. DNA samples (2.5 μl) were then added to the tubes and placed onto a Prime thermocycler (TECHE). Amplification was performed using the following conditions; initial denaturation at 98 °C for 2 min, followed by 40 cycles each com-

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