Original Research Article

Ivoirian molecular profile of high-risk Human papillomavirus: Preliminary study of 250 cases in a single center in Abidjan

ABSTRACT

Background: The carriage of high-risk papillomavirus (HR-HPV) differs between countries and regions of the world. There is not enough data on the genotyping of this virus in Côte d'Ivoire and in Abidjan particularly.

Aim: to illustrate the genotypes of prevalent HR-HPV in the city of Abidjan.

Materials and Method: Descriptive cross-sectional study, carried out over a period of 2 months at the Teaching Hospital of Yopougon. It involved endocervical swabbing of 250 sexually active women. The HPV genotyping was performed by real-time PCR. A logistic regression made it possible to determine the factors associated with the carriage of HPV.

Results: The mean age of the population was 43.33 and a prevalence of 34% was found. HPV 68 was the most prevalent (18.1%) followed respectively by HPV 52 (17.2%), HPV 56 (13.8%), HPV 35 (8.6%), HPV 45 (7.0%). As for genotypes 16 and 18, they only represented (2.5% and 5.1%). The prevalence of multiple infections was (24.6%). The associated factors to the HPV infection were the educational level OR= 0.45; IC 95% [0.24-0.85] and the marital status OR = 0.40; IC 95% [0.20-0.79].

Conclusion: The prevalence of HPV was high, and the genotypes identified are different from those targeted by the currently available prophylactic vaccines. The management with an appropriate vaccine is therefore necessary for these West African countries.

Comment [AaM1]: Delete the brackets

Comment [AaM2]: , respectively

Comment [AaM3]: 24.6%

Keywords: High-risk HPV; PCR; molecular profile; Western Africa

1. INTRODUCTION

Human papilloma viruses (HPV) are small, ubiquitous and resistant viruses. Based on their oncogenic potential in humans, they are distinguished into low-risk HPV, responsible for benign lesions such as condylomata acuminate and warts. Nevertheless also in high-risk HPV is involved in cancers of the anogenital and oropharyngeal sphere. They are also responsible for cervical intraepithelial

dysplasia or neoplasia [1]. HPV genital infection is considered the most common sexually transmitted infection (STI) in the world [2]. The favoring factors of HPV infections are a low socio-economic level [3], early sexual activity, the existence of many sexual partners, immunosuppression (HIV), tobacco, prolonged oral contraception (glucocorticoids, steroid-related compounds found in oral contraceptives) that promote increased transcription and expression of the HPV 16 genome in vitro [3, 4]. Under immunocompetent conditions, the virus is cleared within 12-18 months following the infection. But its persistence (chronicity) is responsible for most cervical cancers [5].

The strong correlation between genital HPV infection and cervical cancer has led to significant biomedical research to understand better its oncogenesis and consider prevention strategies, one of which is anti-HPV vaccination and it occupies an important place. The prophylactic vaccines currently available target only genotypes 6 and 11 responsible for benign genital lesions, and genotypes 16 and 18 found in the majority of cervical cancer cases worldwide, thus allowing a primary prevention [6]. However, the genotypes targeted by these vaccines are sometimes not the most common in some African regions and also the other genotypes at high risk of cancer do not have vaccines available yet. However, these genotypes would be present in the African population. [7], with variations depending on the regions and within the regions themselves as demonstrated in a study carried out in Côte d'Ivoire (Abidjan) [8]. The purpose of this work is to identify the high-risk HPV genotypes prevalent in women in Abidjan, in order to participate in the establishment of an HPV map in the sub-region.

Comment [AaM4]: was

2. MATERIAL AND METHODS

2.1 Study set.

A cross-sectional survey took place from January to March 2017. It first took place in the city of Abidjan, at the Teaching Hospital of Yopougon, specifically in the gynecology and obstetrics department. This site was used to recruit patients for HPV samples. Visual screening sessions for precancerous cervical lesions were carried out over 4 working days a week with an average of 5 to 6 women daily. Biopsy samples, cryotherapy and electroconization were performed immediately

when necessary. Free systematic care adapted to the histological results was offered to the patients. Secondly, the biomolecular analyzes of the samples were carried out in Burkina Faso (Ouagadougou), at the Molecular Biology and Genetics Laboratory of the Pietro Annigoni Biomolecular Research Center, CERBA / LABIOGENE which is a UEMOA center of excellence and a national reference center (DNA extraction, molecular characterization by real-time PCR of HPV) [9].

2.2 *Materials for collecting samples and performing VIA / VIL*

-Materials for genital samples: Sterile cotton-tipped swabs, sterile disposable specula, light source, cotton wool on forceps, clean gloves, cooler and accumulators, acetic acid solution and strong Lugol's solution.

- Materials for viral DNA extraction: 1.5 ml Eppendorf® tubes, micropipettes, vortexes, sterile filter cones or tips, incubator, centrifuge, laminar flow hood.

2.3 Visual examination with acetic acid and Lugol (VIA / VIL)

This screening follows a classic gynecological examination. Thus, after highlighting the cervix with the help of a speculum, a cotton-tipped swab is introduced into the endocervix and the ectocervix at the junction zone and it is rotated at least three times clockwise. Following the endocervical swabbing, the VIA / VIL will always be performed by the healthcare provider and the results reported on a dedicated sheet according to the 2001 Bethseda classification, then archived.

2.4 Human papillomavirus research

2.5.3.1 Viral DNA of the HPV Extraction

The HPV viral DNA extraction was carried out at CERBA / LABIOGENE using the `` DNA-Sorb-A '' kit from SACACE biotechnologies® (reference K-1-1 / A, lot 12F13A150) following the protocol provided by the manufacturer

2.5.3.2 Real-time PCR

The PCR was carried out at CERBA / LABIOGENE using the “HPV Genotypes 14 Real-TM Quant” amplification kit from SACACE biotechnologies® (Ref V67-100FRT Sacace, Italy) which makes it possible to detect fourteen high-risk HPV genotypes. (PVH 16, 18, 31,33, 35, 39, 45, 51, 52,56, 58, 59, 66 and 68).

2.5.3.3 Interpretation of real-time PCR results

Interpretation of results was performed using the Microsoft Excel "PVH 14 Genotypes Real TM.xls" program provided by the manufacturer. The sample result

is invalid in the absence of any fluorescence signal (positive control or internal control). The result is valid if the negative amplification controls do not have a positive fluorescence signal, and if in each of the positive controls the corresponding HPV genotypes are determined. The HPV Genotypes 14 Real-TM Quant kit was able to detect the following 14 high-risk HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. These were recognized according to the type of fluorescence emitted. The sample result is negative if all four tubes contain positive signal only in the first two cells of Cy5 (CI). The result of the sample is positive if the positive signal appears in Fam and / or Joe and / or Rox, and / or in the last two cells of Cy5

2.2 Sample

All women regardless of their age and sexual activity, who gave their free and informed consent to screening during the survey period. Data were collected using a standardized questionnaire. This was a systematic random sampling. Women were gradually included in the study as they were going in for a gynecological consultation, regardless of the reason and as soon as their consent was granted. A code was assigned to each patient's sample to maintain anonymity. The sample thus collected was stored at -20 ° C in the freezer of the biology laboratory of the Teaching Hospital of Yopougon while awaiting shipment. At the end of all the collections, the samples were transported by air to Ouagadougou to CERBA / LABIOGENE for biomolecular analyzes in a cooler generously filled with accumulators in order to maintain the cold chain.

2.3 Variables

The pre-established survey sheets focused on the following qualitative variables (marital status, level of instructions, occupation, place of residence, level of education, recent change of sexual partner, use of condom, contraceptive method,

HIV status, HPV status) and the following quantitative (Age, Age at first sexual intercourse, number of children, number of pregnancies, number of sexual partners and frequency of sexual intercourse).

2.4 Data analysis

For the questionnaires, the data were entered into a database using Epi data software, they were processed and analyzed on a microcomputer using SPSS software in version 20.0. During the bivariate analysis, we used the Chi-square or Fisher test to compare proportions and the averages. The difference was statistically significant for $p < 0.05$. Parameters with a p-value less than 0.20 were included in a logistic regression model. Variables that exhibited collinearity with the dependent variable (the carriage of HPV determined by the PCR)

2.5 Course of the study

2.6 Ethical consideration

Information was collected in the strict confidentiality using an anonymous questionnaire and the results were kept secret. The free and informed consent of each woman involved in the study was obtained after information on the value of the data collected. In addition, they were free to leave the study. Also we had received the agreement of the Ethics Committee for Health Research of Ivory Coast.

3. RESULTS

The average age of the patients was 43.44 years (10.80 CI 42-44.69). Single, unpaid and uneducated women represented 25.74% and 41%, respectively. Women who used condoms often during sex represented 62% of the cases. 36.20% used oral contraceptives for ≥ 5 years. 70.8% of the women had consulted at least once for gynecologic care. HIV seropositivity was found in 3.2% of women (8/250). 37.6% had already been screened for cervical cancer (Table I). The prevalence of women who had VIA and VIL was 18.4% and 27.6%, respectively. There was a 34% high-risk HPV (HR-HVP) co-infection. HPV 68 was the most common (18.10%) in our

study population (Figure 1) and in those with positive VIA / VIL (28.2%) (Table II). HPV 52 and 68 coinfection accounted for 5.7%... . No behavioral and sexual factors were significantly associated with the carriage of HPV. HPV carriage was not associated with the degree of knowledge about cervical cancer. The positive result of VIA and VIL was associated with a significant increase in the risk of carrying HPV. In multivariate analysis, HPV carriage was only significantly associated with education level and celibacy (Table III).

Comment [AaM5]: coinfections

Table I: Socio-professional characteristics, sexual behavior, HIV status and consultation motive of the 250 women.

Variables	Effective (n)	Percentage (%)
Age (years)		
20-30	29	11,6
30-40	83	33,2
40-50	72	28,8
50-60	48	19,2
≥60	18	7,2
Matrimonial status		
In a relationship	186	74,4
Not in a relationship	64	25,6
Level of instruction		
Educated	147	58,8
Uneducated	103	41,2
Socio-professional activity		
Paid	65	26,0
Unpaid	185	74,0
Reproductive value		
Gravidity		
Nulligravida (0)	16	6,4
Primigravida (1)	19	7,6
Pauci gravidae (2-3)	67	26,8
Multigravida (3-4)	100	40
Great multigravida ≥ 5	48	19,2
Parity (n= 234)		
Nulliparous	24	10,2
Primiparous	37	14,8
Pauciparous	95	40,6
Multiparous	56	23,9
Great multiparous	22	9,4
Sexual behavior		
Age of the first intercourse		
Unknown	17	6,8
< 17 years	62	24,8
≥ 17 years	171	68,4
Number of sexual partners		
1	104	41,6
≥ 1	146	58,4
Recent change of sexual partners		
< 1 year	12	4,8
≥ 1 year	238	95,2
Sexual intercourse frequency		
< 3 times / day	241	96,4
≥ 3 times / day	9	3,6
Condoms use		
Always	1	0,4
Sometime	155	62
Never	94	37,6

Oral contraceptives usage for more than 5years		
< 5 years	21	36,2
≥ 5 years	37	63,8
History of gynecological consultation		
Yes	177	70,8
No	73	29,2
Assessment of women's level of knowledge about cervical cancer, HPV and STI		
HIV status		
Positive	8	3,2
Négative	173	69,2
Sero-ignorant	69	27,6
Consultation motive		
Systématic screening	220	88,0
Intermittent metrorrhagia	18	7,2
Pelvial pain	12	4,8

Table II: Appearance of the cervix on visual inspection without preparation, after VIA / VIL and HPV test results

	Effective (n)	Proportion (%)
Without preparation		
Squamocolumnar junction		
Entirely visible	171	68,4
Partially visible	22	8,8
Not visible	57	22,8
Absence de lesion	166	66,4
Cervical polyp	11	4,4
Condyloma	2	0,8
Contact bleeding	18	7,2
Ectropion	32	12,8
Naboth cyst	18	7,2
Budding ulcerative lesion	3	1,2
VIA		
Positive	46	18,4
Negative	204	81,6
VIL		
Positive	69	27,6
Negative	181	72,4
VIA/VIL		
Positive	45	18,0
Negative	180	72
HPV test		
Negative	165	66
Positive	85	34
Number of genotype /patient		
1	64	75,2
2	14	16,4
3	04	04,7
4	03	03,5

Table II: Analytical table of positive HPV contingency and epidemioclinical determinants (IVA / IVL).

	HPV positive n/N (%)	P-value	OR (IC 95%)	*P-value*	Odds Ratio* (IC 95%)
Age					
[20 - 40 years]	38/112 (33,9)		1,07		
] 40 à ≥ 60 years [	47/138 (34,1)	0,807	(0,61-1,85)		
Schooled					
Yes	41/147 (27,9)		0,53		0,45
No	44/103 (42,7)	0,049	(0,29-0,99)	*0,01*	(0,24-0,85)
Profession					
Fixed ressources	17/65 (26,2)		0,813		
Without fixed ressources	68/185 (36,8)	0,578	(0,39-1,68)		
Age at first sexual intercourse					
< 17 years	20/62 (32,3)		0,83		0,87
> 17years	61/171 (35,7)	0,566	(0,44-1,56)	*0,5*	(0,89-3,07)
Number of sexual partners					
< 1 partner	39/104 (37,5)		1,42		
> 1partners	46/146 (31,5)	0,222	(0,80-2,50)		
Condom use					
yes	29/95 (30,5)		0,73		
No	56/155 (36,1)	0,318	(0,39-1,35)		
Duration of oral contraceptive use					
< 5 years	78/229 (34,1)		0,77		
≥ 5 years	7/21 (33,3)	0,946	(0,45-1,34)		
HIV status					
Positive	4/8 (50,0)		2,06		
Negative	57/173 (32,9)	0,318	(0,36-11,30)		
VIA					
Positive	22/46 (47,8)		2,05		0,76
Negative	63/204 (30,9)	0,0284	(1,01-	*0,64*	0,25-2,53

			4,13)		
VIL					
Positive	30/69 (87,0)		1,76		0,87
Negative	55/181 (30,4)	0,0508	(0,95-3,24)	*0,8*	0,33-2,32

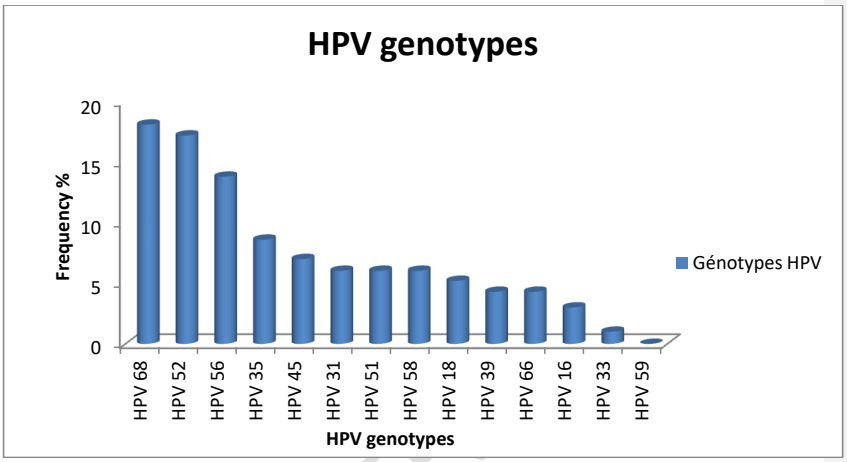

Figure 1: Frequency of the different HPV genotypes detected compared to their total number

4. DISCUSSION

4.1 HPV prevalence and its determinants

In our study the overall circulating HR-HPV prevalence was 34%. The prevalence of HPV infections among women in the general population differs considerably between countries, and regions, ranging from 1.6% to 41.9% [10]. A recent meta-analysis of African publications on the distribution of HPV shows a variable overall prevalence depending on the geographic area. Overall, the prevalence of HPV infections in Africa was higher than in other regions of the world; Indeed, Southern Africa leads with 57.3%, followed by East Africa (42.2%), West Africa (27.8%) and North Africa (12.8%). Our results were similar to the West African region, with Côte d'Ivoire, the study country, being included in the study. These figures also vary among countries in the same region [11]. The high prevalence of HPV infection observed in Africa is believed to be linked to behavioral or sexual factors which

differ from those in Western countries [12, 13]. Indeed, the clinical determinants of HPV infection would be: early sexual activity, the existence of many sexual partners, HIV immunosuppression, tobacco, prolonged oral contraception, sexually transmitted infections (STIs), low socio-economic level [14, 15] These behavioral or sexual factors are found in our study population in variable proportions and were significantly associated with the carriage of HPV only for the level of education and not married As another risk factor we have HIV. Because the prevalence of HR-HPV infection appears to be high in women living with HIV compared to the general population [16-18]. They are more likely to have persistent HPV infection [19] and progression to precancerous lesions (CIN) [20]. However, there is not enough data on the prevalence and distribution of HPV infection in the HIV positive female population [8]. The HIV status was known in 72.4% of cases and only 3.2% of them were HIV positive.

4.2 Distribution and molecular epidemiology of HR-HPV

Most of the studies on the distribution of circulating HPVs was associated with either the pap test (cytology in liquid medium), histology or even the visual screening method (IVA / IVL) [11, 20]. These various genotyping techniques have no impact on the precision of the prevalence of the specific HPV genotype. Indeed, the four most common genotypes, HPV 16, 18, 52 and 33, were identical for both normal cervical cytology and high squamous intraepithelial lesion (HSIL) . HPV 33 was ranked fourth in all regions except in West Africa, where HPV 35 is most prevalent. These results suggest not only the consistency of genotypes 16 and 18, but also a greater prevalence of HPV 45 in invasive cancers and HPV 52 in HSIL [21]. The most frequently encountered HR-HPV genotype in our population was at 18.1% the HPV 68. In our study, HPV 52 came in 2nd position with (17.14%), followed respectively by HPV 56 (13.79%), HPV 35 (8, 62%) and HPV 45 (7%).

HPV 16 and 18, regardless of HIV status or the existence of a cervical defect, had a low prevalence in our study. Meanwhile the majority of the studies revealed a high prevalence in the world and even in Africa [17, 18]. The combined prevalence of HPV 16 and 18 is 67.7%, in agreement with the overall estimation of 70% found by other meta-analyzes [21-23]. Multiple infections accounted for 24.2% in our study population versus 19.8% of multiple infections due to high-risk HPVs in women

under 25 in the Monsonego study. Our result is, however, similar to that of Pannatto, who found a prevalence of 24.3% of multiple infections, all genotypes combined, in women aged 16 to 26 with normal cervical cytology [23]. This heterogeneity of prevalence in the same region would be associated with the age differences of the study populations. However, in general, the prevalence of multiple infections would be higher at a young age. The genotypes most frequently found in multiple infections in our study were HPV 52 (23.1%), followed by HPV 68 (13.5%), HPV 58 (9.6%) with a low prevalence for the 16 and the 18. Our result differs from that found by Panatto, who found that the most frequent genotype in multiple infections was HPV 16 (16%), followed by HPV 52 (12%), HPV 58 (8%), and HPV 56 (6%) [23]. We did not find HPV 16 and HPV 18 Co-Infection, as well as some authors [23-25]. The number of associated genotypes varied from two (2) to four (4) during our study.

4.3 HPV vaccine

The strong mobilization for mass vaccination [26] through the GAVI Alliance, with the aim to fill the gap in terms of vaccines access, was only possible with an agreement signature with the pharmaceutical industries to offer the HPV vaccine at a lower cost for developing countries eligible for the GAVI criteria [8]. Ivory Coast, which has become eligible, should benefit from it from 2018, like 19 other countries in sub-Saharan Africa. The mapping of the HPV infection as presented in our study and those of other regions of Africa poses a problem of adequacy between the genotypes found and the current anti-HPV vaccines. Because modeled on genotypes common in Europe. These results therefore underline the importance of developing new polyvalent prophylactic vaccines taking into account those genotypes frequently observed in these African countries. In fact, the prevalence of HPV 16 and 18 was 2.58% and 5.17%, respectively. So less than 7.7% of the genotypes against 92.3% for all the other HR-HPV in our study. Polyvalent vaccine in phase III clinical trials known to protect against HPV 16, 18, 31, 33, 35, 45, 51, 52, and 58 could prevent almost all cases of cervical cancer in African women. This nanovalent vaccine will not be available before few years in Africa and will therefore leave a whole generation unprotected. These obstacles include the long

period of time it takes for the vaccine to be ready for mass distribution, negotiating its cost, and placing the infrastructure to deliver the vaccine to adolescents.

4.4 Limits

Our study experienced limitations and constraints related in part to the financial cost of identifying HPV genotypes, thus limiting the HPV DNA detection kit that we used to 14 high-risk genotypes instead of about 8 genotypes. The presence of other high-risk HPV genotypes could therefore not be determined in the study population. Furthermore, the nature of the questionnaire (direct questioning of a non-anonymous nature, relating to past informations and their sexual behavior) submitted to the women may have been the source of memorization bias and suggestiveness. As for Selection Bias, they were linked to the place of recruitment which is selective for a particular population: women seeking care. Despite its limitations and constraints, we have the real-time PCR technique which is an advanced technique in the determination of HPV genotypes, since it has a specificity and a sensitivity of 100% each. It is therefore a very fine diagnostic tool which accentuates the scientific value of our study and the results obtained seem important to us in terms of public health.

4. CONCLUSION

The high prevalence found in our study (34%) reflects the socio-demographic, behavioral and sexual characteristics of women. The most frequently found genotype in this study was HPV 68 followed by HPV 52, 56, 35, 45 and 31. Which are not covered by the currently available vaccines. In the wait of the availability of polyvalent vaccines targeting the most common genotypes in most African countries in general and in Ivory Coast particularly, emphasis must be placed on the prevention of HPV infection, by raising awareness and advising responsible sexual behaviors.

Comment [AaM6]: delete this phrase OR correct it to be sentence.

CONSENT (WHERE EVER APPLICABLE)

"All authors declare that 'written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written

consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. **Monsonego J.** Infections à papillomavirus, état des connaissances, pratiques et prévention vaccinale. Paris: *Springer*; 2006.
2. **Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV and al.** Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med.* 2003; 348:518-27.
3. **Hibbitts S, Rieck GC, Hart K, Powell NG, Beukenholdt R, Dallimore N and al.** Human Papillomavirus infection: an anonymous prevalence study in South Wales, *UK. Br J Cancer*. 2006; 95:226-232.
4. **Negrini BP, Schiffman MH, Kurman RJ, Barnes W, Lannom L, Malley K and al..** Oral contraceptive use, human papillomavirus infection, and risk of early cytological abnormalities of the cervix. *Cancer Res*. 1990; 50:4670-5.
5. **Ly A.** Le cancer du col utérin: nouveaux vaccins, nouvelles perspectives? *J Afr Cancer.* 2009; 1:65-7.

Comment [AaM7]: Change this to be: et al. Do the same for all similar words appearing in the references.

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

6. **Anorlu RI**. What is the significance of the HPV epidemic? *Can J Urol*. 2008; 15: 3860-5.
7. **Muñoz N, Méndez F, Posso H, Molano M, Van den Brule AJC, Ronderos M and al**. Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. *J Infec Dis.* 2004; 190:2077-87
8. **Jacquet A, Horo A, Charbonneau V, Ekouevi DK, Roncin L, Toure B and al**. Cervical human papillomavirus and HIV infection in women of child-bearing age in Abidjan, Côte d'Ivoire, 2010. *British Journal of Cancer* .2012. 107,556–563
9. **Bruni l, Barrionuevo-Rosas l, Serrano B, Brotons M, Cosano R, Muñoz J and al**. ICO Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in Burkina Faso. *Summary Report* 2013-12-16. 53p.
10. **Séoud M**: Burden of human papillomavirus-related cervical disease in the extended middle East and north Africa-a comprehensive literature review. *J Low Genit Tract Dis* 2012, 16:106–120.
11. **Ogembo RK, Gona PN, Seymour AJ, Park HS-M, Bain PA, Maranda l, and al.** Prevalence of Human Papillomavirus Genotypes among African Women with Normal Cervical Cytology and Neoplasia: A Systematic Review and Meta-Analysis. *PLoS ONE* 10(4): (2015) Meta Afrik.
12. **Abu-Raddad l, Akala FA, Semini I, Riedner G, Wilson D, Tawil O**: Characterizing the HIV/AIDS Epidemic in the Middle East and North Africa: Time for Strategic Action. In World Bank/UNAIDS/WHO Publication, Middle East and North Africa HIV/AIDS Epidemiology Synthesis Project. Washington DC: *The World Bank Press*; 2010.
13. **Thomas DB, Ray RM, Kuypers J, Kiviat N, Koetsawang A, Ashley RL, and al**. Human papillomaviruses and cervical cancer in Bangkok. III. The role of husbands and commercial sex workers. *Am J Epidemiol*. 2001; 153(8):740-8.
14. **Bleeker MC, Hogewoning CJ, Berkhof J, Voorhorst FJ, Hess-Elink AT, Van Diemen PM and al**. Concordance of specific human papillomavirus types in sex partners is more prevalent than would be expected by chance and is associated with increased viral loads. *J Clin Infect Dis*. 2005; 41:612-20.
15. **Bosch FX, Broker TR, Forman D, and al**. Comprehensive control of human papillomavirus infections and related diseases. *Vaccine* 2013; 31 (suppl 7): H1–31.

Formatted: Highlight

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Highlight

Comment [AaM8]: Write 6 authors, then followed by et al as you did in other references.

Formatted: Highlight

16. **Control CFDA**. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* 1992; 41 (Rr-17): 1–19.
17. **Mcdonald AC, Tergas AI, Kuhn l, Denny l, Wright TC Jr**: Distribution of human papillomavirus genotypes among HIV-Positive and HIV- negative women in Cape Town, South Africa*. Front Oncol,* 2014; 4: 48.
18. **Ahdieh, Klein RS, Burk R, and al**: Prevalence, incidence, and Type-specific persistence of human papillomavirus in human immuno deficiency virus (HIV)-positive and HIV-negative women*. J Infect Dis* 2001; 184: 682–90.
19. **Denslow SA, Rositch AF, Firnhaber C, Ting J, Smith JS** : Incidence and progression of cervical lesions in women with HIV: a systematic global review. *Int J STD AIDS* 2014; 25: 163–77.
20. **Clifford GM, Gallus S, Herrero R, Munoz N, Snijders PJ, Vaccarella S and al:** Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet* (London, England). 2005; 366 (9490):991±8.
21. **Bosch FX, de Sanjose S**. Human papillomavirus and cervical cancer-burden and assessment of causality. *J Natl Cancer Inst Monogr*. 2003; 3-13
22. **Didelot-Rousseau MN, Nagot N, Costes-Martineau V, Vallès X, Ouédraogo A, Konaté I and al**. Human papillomavirus genotype distribution and cervical Squamous intraepithelial lesions among high-risk women with and without HIV-1 infection in Burkina Faso. *British Journal of Cancer*. 2006; 95:355-62.
23. **Panatto D, Amicizia D, Trucchi C, Casabona F, Lai PL, Bonanni P and al**. Sexual behaviour and risk factors for the acquisition of human papillomavirus infections in young people in Italy: suggestions for future vaccination policies. *BMC Public Health*. 2012; 12:623.
24. **Djigma FW, Ouedraogo C, Karou DS, Sagna T, Bisseye C, Zeba M and al.** Prevalence and genotype characterization of human papillomavirus among, HIV-seropositive in Ouagadougou, Burkina Faso. *Acta Tropical*.2011; 117:202-6.
25. **Ouédraogo C, Zohoncon TM, Traoré EMA, Ouattara S, Bado P, Djigma FW, and al**. Distribution of High-Risk Human Papillomavirus Genotypes in Precancerous Cervical Lesions in Ouagadougou, Burkina Faso. (2016) Open *Journal of Obstetrics and Gynecology*, 6, 196-204.
26. **Bedoya AM, Gaviria AM, Baena A, Borrero M, Duarte DF, Combita al.** Age-specific seroprevalence of Human papillomavirus 16, 18, 31, and 58 in women of a rural town of Colombia. *Int J Gynecol Cancer*. 2012; 22(2): 303-10.

Comment [AaM9]: ???

Comment [AaM10]: Write 6 authors, then followed by et al as you did in other references.

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Font color: Red

Comment [AaM11]: ?? Revise it.