Full Length Research Article

WORRYING AND REAL RISK FOR HUMAN PAPILLOMA VIRUS-INDUCED CERVIX LESIONS IN CAMEROONIAN WOMEN: INSIGHT IN A SOCIOECONOMICALLY DISADVANTAGED MUSLIM REGION

[1,*]**Koanga Mogtomo Martin Luther**, [1]**Nkeumacha Ida Patrick**, [1]**Embolo Elisee**, [3]**Banai Thomas**, [1]**Ntatou Lemouchele Idriss**, [2]**Kojom Loick Pradel**, [1]**Assokom Eliane Vanessa** and [1]**Ngono Ngane Annie Rosalie**

[1]Department of Biochemistry, Faculty of Science, The University of Douala, P.O. Box 24157, Douala, Cameroon
[2] Department of Animal Biology, Faculty of Science, The University of Douala, P.O. Box 24157, Douala, Cameroon
[3]Faculty of Medicine and Pharmaceutical Science, The University of Douala, P.O. Box 24157, Douala, Cameroon

ARTICLE INFO

ABSTRACT

Cervical cancer is a real public health problem in developing countries especially in socioeconomically disadvantaged populations which are often very difficult-to-reach by health programs and mistrustful about these. We therefore carried out a pilot hospital-based cross-sectional study in the far north region, one of the poorest regions of Cameroon. This aimed at determining the prevalence of cervical pre-malignant and malignant lesions and of some main risk-increasing factors. Socio-demographical and risk factors for cervical abnormalities were documented. Eye examination of cervix, Papanicolaou smears were set and the results interpreted using the Bethesda system. Mean age of the population was 36.25 ± 10.66 years. Macroscopic analysis revealed abnormalities represented by inflammation (46.7%), bleedings upon contact (40.0%) and budding (13.3%). Abnormal cytology accounted for 11.2 % and was represented by ASCUS (3.8%), ASC-H (1.9%) and LSIL (5.5%).Most of normal-cytology women were at high risk for Human Papilloma Virus infection and its cervical consequences. This pilot study underscored a significant prevalence of precancerous lesions and a clear risk for HPV infection and invasive cancer. Our findings suggest the need for a national program of active case detection of cervical cancer involving health education and prompt management of cases.

INTRODUCTION

Cervical cancer is ranked second among the most prevalent cancer among female through the world. In 2012, an estimated of 528,000 new cervical cancer cases were recorded and it was responsible for about 266,000 deaths of which 70 % in developing countries. In these countries, cervical cancer is ranked first based on estimates related to incidence and mortality (WHO, 2013). For the last three decades, it has been observed increasingly important morbidity and mortality of this female cancer in developing countries on contrary in developed countries where early detection campaigns are consistently implemented and management of cases are optimally efficient and regularly up to date to be the best possible for the patients (WHO, 2007).

*Corresponding author: KoangaMogtomo Martin Luther,
Department of Biochemistry, Faculty of Science, The University of Douala, P.O. Box 24157, Douala, Cameroon.

There is a consensus that the main risk factor for cervical cancer development is persistent infection with high-risk group Human Papilloma viruses (HPVs); together with other factors like multiple sexual partners or human immunodeficiency virus (HIV) infection.HPV is associated with cervical cell modifications that may evolve to precancerous lesions including atypical cell morphology and the different stages of cervical intraepithelial neoplasia or dysplasia (Solomon *et al.,* 2002). Thereafter, an invasive cancer may install after many years throughout which women have no signs or symptoms of the disease (Hantz *et al.,* 2005). In addition, many authors previously outlined women newly diagnosed with cervical cancer or another type of cancer were attending health care facilities at advanced stage of the disease (Pezzatini *et al.,* 2007; Ogembo *et al.,* 2014), jeopardizing more the chance of survival of these patients within their management. Besides, the probability of regression to normal cervical epithelium is inversely proportionate to the stage of the disease

(Riethmuller, 2010). Thus, in consideration of the poor prognostic of cervical cancer as well as the high cost of its management, some authors underscored the need for implementation and scaling up community-based early detection programmes aim to mitigate the deleterious effects of cervical cancer among female population (Koanga *et al.,* 2010; Koanga *et al.,* 2014). In Cameroon, the statistics of cervical cancer are cause of concern and importantly, the some early detection programmes are mainly implemented in some regions especially the towns of Douala (Littoral region) and Yaounde (Center region) respectively business capital and political capital of the country. We therefore carried out hospital-based cross-sectional study in the far north region, one of the most resources-constrained regions of the country. This pilot study aimed at determining the prevalence of different types of the cervical cell alterations and the distribution of main risk factors for HPV infection and consecutive cervical alterations.

MATERIALS AND METHODS

Study setting: This cross-sectional study was a part of a large research project aiming to improve the quality of life of Cameroonian women. The present study was carried out in 2015 at the Mindif district hospital. This hospital has a gynecological ward but unfortunately any cervical screening for cervical cancer is performed. The town of Mindif (10°24'N14°26'E) is located in the division of Mayo-kani (KAELE), sub-division of Mindif in the Far North region of Cameroon, one of the poorest regions of the country. There was a paucity of information about economic and social situation in this region of Cameroon. Nevertheless, a questionnaire-based study, carried out in 2014 by the non-government organization so called ALVT (*Association de Lutte contre les Violences faites aux femmes Extrême-Nord Cameroun*) in collaboration with the University of Maroua issued some information. This study aimed to contribute to the fight for the violence inflicted to girls and women and based on its results, the Far North, North and Adamoua regions account for 45 % of all cases of forced and early marriage. Girls aged between 13-15 years old are the main victims of forced and early marriage and have also poor level of education (most have no formal education or have been dropped out of school) owing to the poverty of their families. The main causes of early and forced and early marriage were: socio-cultural practices (41 %), poverty (31 %) and the lack of knowledge about laws on protection of under age (16 %)(ALVF, 2014). Thus, women from the Far North region would beat high risk of HPV infection and consequently precancerous and cancerous lesions. To the best of our knowledge, we did not found any study about cervical abnormalities in women in this area. Therefore, our investigations and findings consequently would be pioneers in this area of Cameroon.

Study population: The study participants consisted of women attending the Mind if district hospital without regard to their age, having sexual activity, willing to participate in the study and signing the informed consent forms. On the other hand, women having no sexual activity, having undergone a total hysterectomy, in menstruation period, under vaginal treatment and having not signed the informed consent forms were excluded from the study. A total of 58 women aged 19-61 years old were enrolled in the study.

Study design: Women were randomly recruited at the Mindif district hospital with respect to aforementioned inclusion and exclusion criteria. If willing to participate in the study a structured questionnaire form was administered to them for 10-15 minutes through standardized interview. Thereafter macroscopic analysis of cervix and cervical epithelium sampling for Pap smear as previously described were performed (Hawes *et al.,* 2003).The first part of the questionnaire was concentrated on demographic variables which included age, community of residence, level of education and marital status. The second part was designed to capture risk factors for cervical alterations such as the number of sexual partners, oral contraceptive usage, parity, alcoholism, age at the first pregnancy. Risk for HPV infection and cervical cancer was defined as at least one the following: (a) early sexual activity (age at first intercourse and first pregnancy); (b) poor level of education or unawareness about HPV infection; (c) multiple sexual partners; (d) polygamy regimen; (e) consumption of alcoholic beverage; (f) oral contraceptive usage; (g) Multiparous or grand multiparous. Results of Pap smear were interpreted and classified using the Bethesda system. It enables to differentiate between negative result, atypical squamous cells of undetermined significance (ASCUS), LSIL, HSIL and invasive cervical cancer (Solomon *et al.,* 2002). Slides were read by a skilled pathologist of the *Centre Pasteur du Cameroun* located in Yaounde (Yaounde, Center region, Cameroon).

Ethical considerations

This study was carried out in conformance with the guidelines for human experimental models in clinical research as stated by the Cameroon Ministry of Public Health and the Helsinki declaration. Besides, the ethical and administrative clearances for this study were issued by the national institutional review board of the Cameroon government (2014/08/485/ CECNERSH/SP). The aim and objectives of the study were explained to them in the language they understood best (French or English), and their questions were answered. Only women who signed an informed consent form for their participation were enrolled. Participation in the study was strictly voluntary and women were free to decline answering any question or totally withdraw if they so wished at any time. Furthermore, there was no difference in the cancer related care provided to women who accepted to participate in the study and those who did not.

Statistical analyses

All data were verified for consistency, coded, and keyed in an Excel sheet. Thereafter, statistical analyses were performed with Graphpad prism 5.0 for windows. Data were summarized in table as percentages with 95% confidence interval (95%CI) or mean ± standard deviation (SD) for qualitative and quantitative variables respectively where appropriated. Goodness-of-fit and independence chi squares (χ^2) were used to compare proportions (univariate analysis). A *p-value*< 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the participants: In total, 58 women were included in the study. They were aged between 19 and 61 with a mean age of 36.25 ± 10.66 years.

Table 1. Prevalence of risk factors of HPV infection in women with normal cytology

Variables (N = 48)	Categories	Frequency	Percentage (95%CI)	P-value
Age (years)	[15-30[	15	31.3 (19.1 - 46.4)	0.0680
	[30-45[	23	47.9 (33.5 - 62.7)	
	≥ 45	10	20.8 (11.0 - 35.4)	
Level of education	None	12	25.0 (14.1 - 39.9)	< 0.0001
	Primary	24	50.0 (36.7 - 63.3)	
	Secondary	9	18.8 (9.4 - 33.1)	
	University	3	6.2 (1.6 - 18.2)	
Marital status	Single	2	4.2 (0.7 - 15.4)	< 0.0001
	Married/Monogamy	36	75.0 (60.1 - 85.9)	
	Married/Polygamy	10	20.8 (11.0 - 35.4)	
Age at the first intercourse (years)	Less than 15	5	10.4 (3.9 - 23.5)	< 0.0001
	15 and older	43	89.6 (76. - 96.1)	
Age at the first pregnancy (years)	Less than 20	28	60.9 (45.4 - 74.6)	0.1400
	20 and older	18	39.1 (25.5 - 54.6)	
Number of sexual partner	One	42	87.5 (74.1 - 94.8)	< 0.0001
	Several	6	12.5 (5.2 - 25.9)	
Use of contraception	No	38	79.2 (64.6 - 89.1)	0.0830
	Yes	10	20.8 (11.0 - 35.4)	
Alcohol beverage intake	No	28	58.3 (43.3 - 72.1)	0.1400
	Yes	20	41.6 (27.9 - 56.7)	
Gravidae	1-3 (Paucigravidae)	17	35.5 (22.6 - 50.6)	0.2570
	4-7 (Multigravidae)	11	22.9 (12.5 - 37.5)	
	≥ 8 (Grand multigravidae)	20	41.6 (27.9 - 56.7)	
Number of abortion	None	30	62.5 (47.3 - 75.7)	< 0.0001
	One	12	25.0 (14.1 - 39.9)	
	Two	6	12.5 (5.2 - 25.9)	
Parity	1-3 (Pauciparous)	20	41.6 (27.9 - 56.7)	0.0080
	4-7 (Multiparous)	11	22.9 (12.5 - 37.5)	
	≥ 8 (Grand multiparous)	17	35.5 (22.6 - 50.6)	

Data are presented as frequency and percentage; 95%CI = confidence interval at 95%; HPV = Human Papilloma virus
Categorical variables were analyzed by the Goodness-of-fit chi square (χ^2) test. P-values < 0.05 are considered significant.
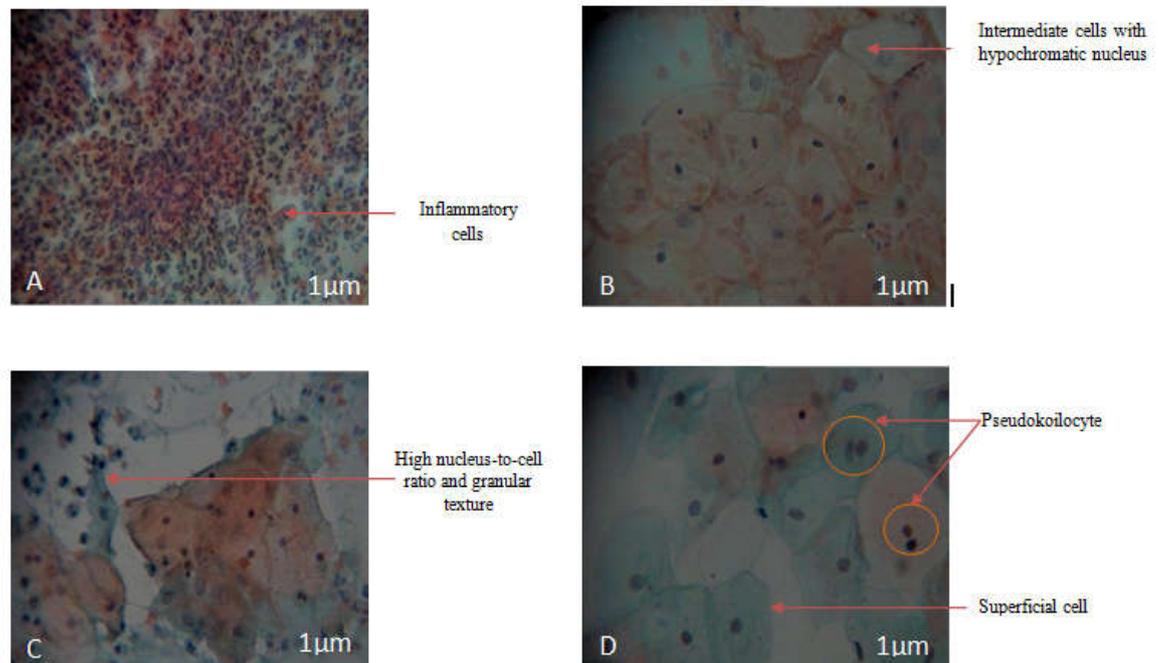

Fig 1.Interpretation of thin-smear based cervical cytology according to the Bethesda system. A: inflammatory cells; B: ASCUS lesion (cells presenting hypochromatic nucleus); C: ASC-H lesion (in basal cells presenting high nucleus-to-cell ratio and granular texture); D: Low-grade squamous cells intraepithelial lesions (LSIL).ASCUS = Atypical squamous cells of undetermined

Most of patients were aged 30-45 years (30, 51.7 %) and then followed by 15-29 years (16, 27.6 %) and 45 and older (10, 20.7 %) age groups respectively. With regard to the level of education, 4 (6.9 %) had attended university, 10 (17.2 %) secondary, 29 (50.0 %) primary and 15 (25.9 %) had no formal education at all (p-value < 0.001). Overall, 55 (94.4 %) were married while 3 (5.6 %) were single (p-value < 0.001).

Macroscopic analysis results

A macroscopic analysis of cervix was performed prior to the processing and understanding the Pap slides. Out of 58 women in this study, 15 (25.9 %) have presented macroscopic cervical abnormalities. These included cases of inflammation (7, 46.7 %), bleedings upon contact (6, 40.0 %) and budding (2, 13.3 %) (χ^2 = 1.625; df = 2; p-value = 0.4440).

10525

Koanga Mogtomo Martin Luther et al. Worrying and real risk for human papilloma virus-induced cervix lesions in cameroonian women: insight in a socioeconomically disadvantaged muslim region

Prevalence of cervical alterations

Four specimens were excluded from this analysis because defined as "unsatisfactory" in accordance with the Bethesda System. Indeed, more than 75.0 % of epithelial cells were obscured by inflammatory cells as depicted in the figure 1. So, following results are presented for 54 patients. The Pap smear test results revealed the following within the study cohort: those with normal cervical cytology (48/54, 88.8%, 95%CI: 76.7-95.4) and those with abnormal Pap smear; ASCUS (2/54, 3.8 %, 95%CI: 0.6-13.8), ASC-H (1/54, 1.9 %, 95% CI:0.1-11.2) and LSIL (3/54, 5.5%, 95%CI: 1.5-16.4) respectively. These different observations are also depicted in the figure 1.

Prevalence of risk factors

The distribution of some main risk factors for HPV infections and invasive cancer in women having presented a normal cytology are summarized in the table 1. Overall, the risk factors were obviously prevalent among this cohort. Indeed, an important proportion was aged 30-45 years (47.9 %) (P-value = 0.0680), had a poor level of education (25.0 %) (p-value < 0.0001), gave young birth the first time (60.9 %) (P-value = 0.1400), had multiple partners (12.5 %) (p-value < 0.0001), were using contraceptive mean (20.8 %) (P-value = 0.0830), were consuming liquors (41.6 %) (P-value = 0.1400) and were multiparous (22.9 %) and grand multiparous (35.5 %) (P-value = 0.0080).

DISCUSSION

Early detection of cervical abnormalities is a helpful and cost-effective strategy for preventing pre-invasive lesions and invasive cancer especially in resources-constrained countries. This study aimed to determine the prevalence of cervical abnormalities among women in a region dramatically poor of Cameroon and the prevalence of some main risk factors. More than one quarter of women enrolled in this study had macroscopic cervical abnormalities which included inflammation, bleedings upon contact and budding. These could be explained by existing microbial infection, physical or chemical agents. In this study microbial causes were not looked for in this study. Even so, we previously demonstrated that yeast germs as *Candida albicans*, bacteria germs and virus as HPV and Herpes simplex virus type 2were associated with these abnormalities (Koanga *et al.,* 2014; Koanga and colleagues, University of Douala, Cameroon, personal communication). Importantly, more than half of these women were using contraceptive method (data not shown). Thus, physical and chemical stress induced by this practice could likely explain one part of the macroscopic abnormalities founded in this study. Cervical abnormalities, following the cytological analysis, accounted for 11.2 % among women included. Our results are similar to those by Tebeu et al. (2005), Mbu *et al.* (2008) and Koanga *et al.* (2014) who founded 7.9 %, 8.3 % and 10.5 % respectively. Differences in sample size, study design, study area may explain these discrepancies. We also noted most of patients with abnormal Pap slide were at risk with respect to the aforementioned risk factors despite none of differences were significant. This may be explained by the small size of our study sample. Worrisome, all women included in this study confessed never undergone a cervix examination before our study and had no knowledge about cervical cancer or HPV. Many reports outlined an advanced stage in women diagnosed with cervical cancer because of late presentation at the hospital. This late presentation is partly due to a poor awareness about HPV and cervix cancer (Pezzatini *et al.,* 2007; Koanga *et al.,* 2010; Ogembo *et al.,* 2014).The cases of cervical abnormalities in this study were represented by ASCUS, ASC-H and LSIL only. All being equal elsewhere, these women would likely be presented at an advanced stage of the disease if study as ours had not been implemented and then complicated their prognosis one time under treatment at the point of care. Thus keeping all these on mind, community or hospital-based early detection programs involving information-education-communication (IEC)-based campaigns about HPV and cervical cancer are needed at the nation level and especially in resources-limited and difficult-to-reach areas.

There is now a consensus about some factors recognized as increasing the risk for HPV infection. These factors include poor level of knowledge about HPV and cervix cancer, level of education, number of sexual partners, parity, use of condoms, early sexual activity, age, smoking and alcoholism(Murthy and Mathew, 2000; Tebeu *et al.,* 2008; Koanga *et al.,* 2010). In our study, most of patients with normal cytology were at high risk for HPV infection and consequently for pre-invasive lesions and malignant progression. Indeed, they mainly were aged 30-44 years old, had a poor level of education, had sex at early age (less than 15 years) and were multiparous (Table 1). Some authors pointed out the frequency of sexual activities are important in women within this age group, therefore increasing the chance for sexually-transmitted infections as HPV infection (Meston *et al.,* 2010).This assumption is reinforced by the fact that in this study the majority of recorded cervical abnormalities were more frequent in women fitting in this age group (data not shown). As regard to intercourse at early age, we must noted that throughout adolescence cervix cells are hallmarked by a low level of differentiation and a high multiplication rate which may select for malignant secondary transformation following to sexual activity-related traumatism (Bergeron, 2008). These malignant transformations are also frequent in cylindrical cervical epithelium which is frequently exposed (phenomenon so called as "eversion") to chemical or physical traumatism in multiparous women (Giraud *et al.,* 1997). The poor level of education is strongly associated with the poor knowledge and misconceptions about HPV infection and consequently translated into risk-increasing sexual behaviors (Tebeu *et al.,* 2008). These authors founded a high rate of unawareness (72.0 %) about HPV in Cameroonian women in Maroua, another town located in the Far North region and close to our study area. Our findings imply the implementation and the scaling up health education programs again. The main limitation of this study is the small sample size undermining the possibility to conveniently extend our findings to the general population. Besides, others main risk factors such as HIV infection have not been investigated. Nonetheless, this pilot study provides some interesting findings necessary to delineate some key points for a possible health program in this region.

Conclusion

This study underscored a clear risk for HPV infection, precancerous lesions and invasive cervical cancer. Our findings suggest the need for a national program of active case detection of cervical cancer involving health education and prompt management of cases. This is necessary in order to mitigate the burden inflicted by cervical cancer among women.

Such objective compulsorily requests a deep implication of all stakeholders involved in the public health field including policies makers, clinicians, researchers and population.

REFERENCES

ALVF, 2014. [Reference study about early and forced marriage in Cameroon, 2014]. Association for fighting against violence make to women native from the Far North region. 2014.p. 2.

Bergeron, C. 2008. [HPV and cancer : Classification of lesions] *Rev. Franc. Lab.,* 2008 (405) : 43-50.

Giraud, J.-R., Coiffic, J., Poulain, P.and Kerisit,J. 1997.[Interest of searching for intraepithelial lesions of cervix in women treated for adnexitis]. *J. Gynecol. Obstet. Biol. Reprod.,* 26: 798-803.

Hantz, S., Alain, S. and Denis, F. 2005. [Anti-HPV vaccines and prevention against cervix cancer]. *Press. Med.,* 34:7453-7457.

Hawes, S.E., Critchlow, C.W., Faye Niang, M.A., Diouf, M.B., Diop, A., Toure, P., Aziz K.A., Dembele, B., Salif,S.P., Coll-Seck, A.M., Kuypers, J.M. and Kiviat, N.B. 2003. Increased risk of high-grade cervical squamous intraepithelial lesions and invasive cervical cancer among African women with human immunodeficiency virus type 1 and 2 infections. *J. Infect. Dis.,* 188:555-563.

Koanga, M.M.L., NgonoNgane, A., Djiakam, N.G., Wankam, M., Brulet, Epaka, C. andAmvamZollo, P.H. 2014. Association of Cervical Inflammation and Cervical Abnormalities in Women Infected with Herpes Simplex Virus Type 2. *Int. J. Trop. Med. Publ. Health.,* 1 (1): 1-4.

Koanga, M.M.L., NgonoNgane, A., Wandjis, A., Dongang, Nana, R.R., Donfack,T.H., Nganwa, G., Wankam, M. andAmvamZollo, P.H. 2010. Sexual behaviour: human papilloma virus and cervical cancer riskamong university students in Cameroon. *Afr. J.Haematol.Oncol.,* 1(4):115-121.

Mbu, E.R., Kongnyuy, E.J., Mbopi-Keou, F.X., Tonye, R.N., Nana, P.N. and Leke, R. 2008. Gynaecological morbidity among HIV positive pregnant women in Cameroon. *Reprod. Health.,* 5:3.

Meston, C.M., Hamilton, L.D. and Harte, C.B. 2010. Sexual motivation in women as a function of age. *J. Sex Med.,* 6 (12): 3305-3319.

Murthy, N.S. and Mathew, A. 2000. Risk factors for pre-cancerous lesions of the cervix. *Eur. J. Cancer Prev.,* 9 (1): 5-14.

Ogembo, J.G., Muffih, T.P., Maranda, L., Amai, R.W., Kemunto, R., Welty, E., Welty, T., Manga, S., Bradford, L., Kalmakis, K. and Sheldon, L.K. 2014. Cervical Cancer in Cameroon: A Three Pronged Approach to Increase Awareness, Vaccination, Screening and Treatment (poster), pp. 1-4.

Pezzatini, M., Marino, G., Conte, S. andCatracchia, V. 2007. Oncology: a forgotten territory in Africa. *Ann. Oncol.,* 18: 2046 - 2047.

Riethmuller, D. 2010. [Human papilloma virus infection : gynecological aspects]. *Mt. Ped.,* 13 (1): 43-49.

Solomon, D., Davey, D., Kurman, R., Moriarty, A., O'Connor, D., Prey, M., Raab, S., Sherman, M., Wilbur, D., Wright, Jr. T., Young, N. and for the forum group members and Bethesda 2001 workshop. 2002. The 2001 Bethesda system: Terminology for reporting results of cervical cytology. *JAMA,* 287 (16): 2114-2119.

Tebeu, P.M., Major, A.L., Rapiti, E., Petignat, P., Bouchardy, C., Sando, Z., De Bernis, L., Ali, L. and Mhawech-Fauceglia, P. 2008. The attitude and knowledge of cervical cancer by Cameroonian women; a clinical survey conducted in Maroua, the capital of far North province of Cameroon. *Int. J. Gynaecol. cancer,* 18(4): 761-765.

Tebeu, P-M., Sandjong, I., Nkele, N., Fokoua, S., Achu, P., Kouam, L. andSamaDoh, A. 2005. [Precancerous lesions of cervix in rural setting: a cross-sectional study]. *Med. Afr. Noire.,* 52 (1): 27-31.

World Health Organization, 2007. Fight against cervix cancer. General Guidelines of essential practices. World Health Organization, Switzerland, *Geneve.* 2007.p.124.

World Health Organization. International center for research on cancer. Last world estimates on cancer. N° 223 Press report. 2013.p.3.

****