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Original Research Article

Dysfunctional endometres in Libreville: 20 years retrospective study

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ABSTRACT

Background: The objectives of this work were to inventory the different morphological entities, to alert the medical class to the need for multidisciplinary care; to sensitize the health authorities on this pathology having a considerable influence on the fertility of the woman and a possible evolution towards cancerous disease.
Methods: A retrospective study was carried out over 20 years from January 1983 to December 2002 at the laboratory of anatomy pathology of the faculty of health sciences in Libreville. The revealing clinical signs were the couple's bleeding and infertility. Curettage, endometrial biopsy, and subtotal or total hysterectomy were fixed with 10% buffered formalin or Bouin's fluid. After staining, histological study was carried out by the pathologist.
Results: The histological images of the endometrial polyps were the most representative (73 cases or 17.68%), followed by prolonged proliferative endometers (70 cases, or 16.95%), glandulo-cystic hyperplasia (66 cases, or 15.98%) and persistent proliferative endometers (58 cases, or 14.04%). More than half of the numbers concerned women under 35 (225/413 cases); in those over 45 years of age, the predominantly endometrial carcinoma was found. The most affected province was Woleu-Ntem (24%), followed by Haut-Ogooué (18%).
Conclusions: The different dysfunctional endometers are also described in the different regions of the continent. The management of these aims to be multidisciplinary and urgently requires a frank collaboration between clinician and morphologist, especially for country where a birth policy is clearly expressed by governing bodies.

Keywords: Retrospective study, Histological study, Dysfunctional endometers, Endometrial polyps

INTRODUCTION

Endometrium refers to any abnormality of the endometrium that is reversible spontaneously or after replacement therapy or secondary to inadequate secretion of ovarian hormones. Indeed, any abnormality in the secretion of these hormones prevents or delays the function of the corpus luteum. The hormonal disorder can be located at the hypothalamic-pituitary (FSH-LH) level and/or at the level of estradiol or progesterone secretion.[1,2]

In Gabon, radio immunoassay of hormones is very expensive. This is why the histopathological study of the endometrium by aspiration or biopsy is commonly requested, to assess the hormonal dysfunction, which causes dysfunctional endometria.

Our work aims to make our modest contribution to identify the histological images of dysfunctional endometria, encountered at the pathological anatomy laboratory of the faculty of medicine of Libreville over the past twenty years (1983-2002).

This is a preliminary work which concerns an important entity of gynecological pathology in Gabon.

Indeed, this country with an area of 274,000 km^2 for a population of 1,350,000 inhabitants, needs more than any other to increase the number of its population. As such,

we can consider dysfunctional endometria as an integral part of the major factors of subfertility observed in Gabonese women, just like the sexually transmitted infections responsible for tubal obstructions.

METHODS

Ethical considerations

This study was reviewed and approved by Gabon's national research ethics committee (NRC). No name has been written on the pre-established data sheet to ensure confidentiality and all records have been kept secure.

Places, type and period of study

We carried out our work at the laboratory of anatomy pathology of the faculty of medicine of health sciences (FMSS) of the university of health sciences (USS) in Libreville. This is a retrospective study spanning a 20-year period from January 1983 to December 2002.

Study population, inclusion and exclusion criteria

The consultation of the registers made it possible to select the dysfunctional endometrial files. In the files consulted, the following were recorded: civil status data (name, sex, date and place of birth and histological file number); socio-demographic data (nationality, race and geographical origin, current place of residence, profession, marital status, name of attending physician and structure of origin); clinical and paraclinical data (date of last normal menstruation, date of symptom onset, patient history, functional signs, organ removed, type of sample (biopsy/excision), radiological, ultrasound and hormonal data); therapeutic data (type of treatment received, side effects and doses of treatment received).

As inclusion criteria, we retained any material for curettage, endometrial biopsy or hysterectomy done for unexplained bleeding. We therefore excluded all cases of spontaneous or induced abortion and those for which the liaison form was incorrectly completed. Thus, from these files, we analyzed histological aspects of dysfunctional endometres encountered during this period in Gabon.

Histopathological study

The samples (biopsies and excision pieces) were sent to the pathological anatomy laboratory where they underwent a first treatment by the different stages of the classic histology technique.[3] Briefly, the samples were fixed in 10% formalin, the fixing time was variable and the amount of fixative used was 10 times greater than the volume of tissue to be fixed. The fragments were then incubated in cassettes which underwent dehydration and then inclusion in paraffin. The inclusion was carried out using an automaton, called histokinette. The 3-5 µm sections were made with a rotary microtome. These bowls were spread out by unrolling them on the slide by flotation on the surface of a hot bath. The slides were placed in an oven at 70°C for 1 hour, then dipped in toluene to remove paraffin from the tissue section so that the dyes could penetrate the tissue. The slides were then passed through a running water bath to replace the alcohol with water. Hematoxylin and eosin were used for histological staining to differentiate all the elements of a tissue. The aim is to highlight the nuclei, the cytoplasm of the cells and the collagen fibers, the hematoxylin colors the nucleus in purple, the eosin colors the cytoplasm in pink and the saffron colors the collagen fibers in yellow. After staining, the glass slides were fixed to the tissue section using a mounting medium (toluene) to provide mechanical and chemical protection of the sections. Slides were examined under a microscope.

Statistical analysis

The data collected was entered on an excel file (Microsoft office 2007), then imported on the stat view software (version 5.0) and the statistical package for social science software (SPSS) (version 10.1) for statistical analysis. The data were divided into qualitative and quantitative variables: the results of the qualitative variables (nominal) are given in numbers (n) and percentages (%); quantitative variables were averaged; statistical significance thresholds were considered for $p<0.05$.

RESULTS

Of all the samples received in the anatomy-pathology department from January 1983 to December 2002, 413 cases of endometrial samples were collected.

Regarding the type of material, sampling by curettage was the most representative (87.65%) followed by hysterectomy (9.93%). As regards the histological type, out of 413 cases of endometrial samples, endometrial polyps were the most representative (73 cases or 17.68%), followed by prolonged proliferative endometria (70 cases, or 16.95%), glandulocystic hyperplasia (66 cases, or 15.98%), persistent proliferative endometers (58 cases, or 14.04%) and finally irregularly secretory endometres (55 cases, or 13.32%). It should be noted that there were 41 cases of endometrial cancer or 9.93% (Table and Figure 1).

Regarding the clinical picture, hemorrhages and sterelities are most revealing symptoms with respectively 303 cases (73.35%) and 106 cases (25.66%) (Table 1).

The distribution according to age indicates, in general, that the age group most affected is that of 26 to 35 years with 156 cases or 37.77%, then that of 36 to 45 years with 106 cases or 25.66. % (Table 2).

Endometrial polyps (29 cases), irregularly secretive endometria (29 cases), prolonged proliferative endometers (28 cases), glandulocystic hyperplasia (28 cases) and persistent proliferative endometers (26 cases)

are more representative in the section of 26 to 35 years old. However, endometrial cancers are more prominent in the age group over 45 years (Table 2).

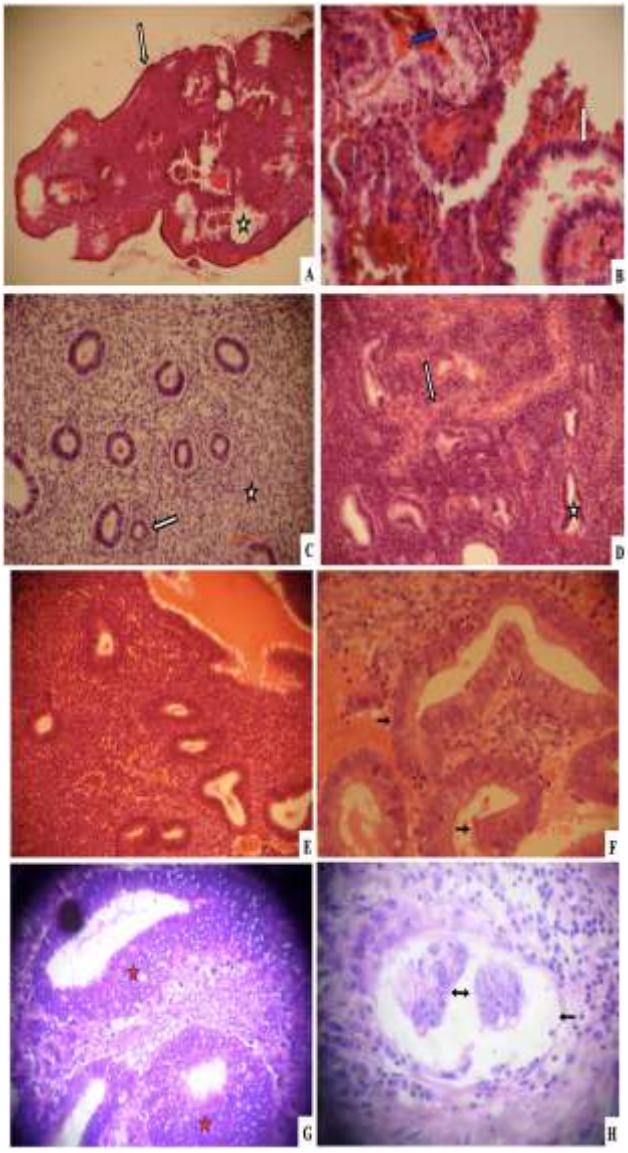

Figure 1: Dysfunctional endometrium.

A: Endometrial polyp intact surface coating (⇩) and cystic-prone glands (☆) X100 HE. **B**: Irregularly secretory endometrium (▬) gland with secretory coating and gland with proliferative coating (⇩). **C**: Dragging regenerative endometrium (small, evanescent gland ⇨, immature and spindle-shaped chorion ☆). **D**: Prolonged proliferative endometrium (proliferative glands ⇨ and arterioles with hyalinized walls ☆). **E**: Endometrium in benign glandulocystic hyperplasia (covering of the glands without atypia). **F**: Endometrium with atypical glandular hyperplasia (lining of the dysplastic glands and with cyto-nuclear atypia (★) backing of the glands (▬). **G**: Endometrial carcinoma developed on an atypical glandular hyperplasia for which hysterectomy has been requested (gland whose lining is neoplastic (★). **H**: carcinomatous lymphangitis (▬) found in the part of total hysterectomy. Note two clusters of neoplastic cells in the lymphatic vascular lumen (▬).

Table 1: Percentage of different types of material, histological type identified and clinical pictures found.

Variables		Effective	%
Types material	Suction	10	2.42
	Curettage	362	87.65
	Hysterectomy	41	9.93
	Total	413	100
Histological types	Endometrial cancer	41	9.93
	Endometrium not very active	9	2.18
	Prolonged proliferative endometrium	70	16.95
	Persistent proliferative endometrium	58	14.04
	Regenerative trailing endometrium	27	6.54
	Endometrial irregularly secretory	55	13.32
	Glandulocystic hyperplasia	66	15.98
	Atypical hyperplasia	14	3.39
	Endometrial polyp	73	17.68
	Total	413	100
Table clinic	Sterility 1 or 2	106	25.66
	Hemorrhage	303	73.35
	Amenorrhea	4	0.96
	Total	413	100

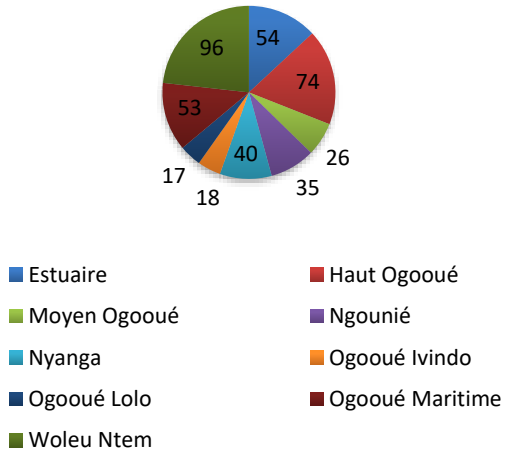

Figure 2: Regional origin of patients.

All provinces of Gabon are affected by this pathology, the most represented are: Woleu Ntem (96 cases, 23%), Haut Ogooué (74 cases, 18%), Estuary (54 cases, 13%) and Ogooué Maritime (53 cases, 13%) (Figure 2).

Table 2: Age distribution of the different histological types of dysfunctional endometrium.

Slices age (years)	9 to 25	26 to 35	36 to 45	>45	Total	Percent (%)
Endometrial cancer			2	39	41	9.92
Endometrium not very active	1	3	5	0	9	2.18
Prolonged proliferative endometrium	21	28	17	4	70	16.95
Persistent proliferative endometrium	10	26	12	10	58	14.4
Regenerative trailing endometrium	4	7	14	2	27	6.54
Endometrial irregularly secretory	12	29	14	0	55	13.32
Glandulocystic hyperplasia	7	28	14	17	66	15.98
Atypical hyperplasia	1	6	7	0	14	3.39
Endometrial polyp	13	29	21	10	73	17.68
Total	69	156	106	82	413	100

DISCUSSION

Dysfunctional endometres that may or may not be associated with pregnancy or hormonal therapy can be analyzed using a logical approach.[4] In our regions, where hormonal dosages are not readily available, pathological examination is a reliable examination that is simple and inexpensive to perform.[5] Of the 413 cases collected in the study, over twenty years, we found that a number of 331 cases concerned women of childbearing age. This is to say the interference of this pathology with the reproductive function of women. Our figures overlap with those of Sakande et al who carried out a retrospective study of endometrial biopsies at CHU Yalgo Ouagadougou in Burkina-Faso in 1998, as well as those of Mbakop in Cameroon.[6,7]

In the present study, several histological types of dysfunctional endometrium were identified, in particular: Endometrial polyps, prolonged proliferative endometers, glandulocystic hyperplasia, persistent proliferative endometers, irregularly secretory endometers, endometrial cancers, trailing regenerative endometers, atypical hyperplasia and inactive endometers (Figure 1).

For endometrial polyps, in general the polyp is of large size, average of the material measuring 8 cm of major axis in clusters.[8,9] There is no connection with endometrial cancer. Its hamartomata's origin is supported by some authors.[10] It is often revealed by bleeding in women whose average age is between 26 and 35 years.[11-14] Its diagnosis is usually not a problem for the histopathologist. In fact, the axis of the polyp is rich in spiral arterioles with a thickened and hyalinized wall, while the sometimes-cystic acorns adopt a radial arrangement. Hertig et al underline their preferential occurrence in young infertile women.[15]

For prolonged proliferative endometers, these are endometers whose general appearance is reminiscent of an advanced proliferative phase, however, possessing a mature stroma. In the literature, this entity is correlated with the persistence of an inadequate follicle or more frequently an inadequate young body formed late.

For granulocytic hyperplasia, this type of dysfunctional endometrium has already been the subject of previous work.[16] There is no connection with endometrial cancer because the proliferation has already stopped or is in the process of spreading. The glands tend to become cystic.[17,18]

Regarding persistent proliferative endometers, with a number of 58 cases, or 14.04%, are found in women whose age range varies from 26 to 35 years, whereas this is observed during the perimenopausal period in the West.[19] The chorion of this type of endometrium is sometimes mature, contains non-spiral arterioles. The glands are provided with a proliferative-type covering of varying size, sometimes diverticular. They include sub-nuclear secrets per field. This lesion evolves on the same ground as that of endometrial adenocarcinoma but the filiation has not been demonstrated despite a climate of hypoestrogenism.

With regard to irregular secretory endometria, this is a lesion encountered 50 times, or 13.32% of cases, with a peak in frequency in women aged between 26 and 35 years. This type of dysfunctional endometrium is characterized by dancing, contracted, menstrual-like stroma.[20] The glands generally have a proliferative, rarely secretory coating, in this case with apical vacuoles. Philippe et al have been correlated with sporadic ovulation or ovulation occurring after numerous monophasic cycles.[21]

For endometrial cancers, are dominated in Gabon with adenocarcinoma, are almost always found in elderly women often in postmenopause.[22-24] The affiliation with atypical hyperplasia is mentioned in the literature, especially in the context of hyperestrogenia.[19-25] Unlike cervical carcinoma, early detection is currently not possible. The lesion is sometimes asymptomatic, however, with metrorrhagia accompanying this antity.[26]

For trailing regenerative endometers, its number in our series is 27 cases or 6.54%, predominant in women aged 36 to 45 years. The lesion is characterized by an endometrium whose stroma has a loose structure, rich in spindle cells. The small-caliber glands appear to be effacing, sometimes despite the presence of infra-nuclear vacuoles. According to Philippe et al this lesion generally results from sub-involution of the corpus luteum with delayed estrogen recovery following abortion or prolonged progestin therapy.[21]

For atypical hyperplasia's, they have also been widely addressed by the work of Mandengou.[16] It corresponds to a precancerous lesion and must therefore be followed with extreme attention.[5] It is true that spontaneous regression has been reported but the affiliation with endometrial carcinoma is widely suggested in the literature.[19,27-30] In our series, the lesion is found at all age groups with a peak between 36 and 45 years. Morphologically, there is a dystrophy of the glands arranged in a disorderly manner, leaning against each other on a certain microscopic field. The lining of the glands is often of the proliferative type. The terrain is the same as that of endometrial adenocarcinoma. The fragility of the doctor-patient relationship does not allow us to be positive about the spontaneous benign evolution of this type of lesion.[31]

Finally, for endometrials that are not very active, with a number of 9 cases (2.18%), this lesion is found in the present study in women aged 36 to 45 years. Morphologically, the image is reminiscent of an endometrium that is difficult to date, where there is a harmonious development of the glands and the stromatus. The spiralization of the arterioles barely outlined reflects luteal insufficiency.[32]

The problem of dysfunctional endometria in Gabon is almost surely overshadowed by that linked to the affections of the uterine tubes. Indeed, the pressure exerted by secondary sterelity where tubal involvement is automatically suspected, such that the practitioner only belatedly thinks of exploring the state of the endometrium. This is why, to better illustrate this point, we bring you the story of a young couple. This is only an invitation to a frank collaboration between morphologist and clinicians for the exclusive benefit of the patients to whom all our actions are in principle dedicated. Madame M. A. C is a young lady born 15/10/1976, Gabonese, administrative officer in the public service of the country. His medical and surgical history is unremarkable. On the obstetrical level, there was a spontaneous abortion at six weeks of amenorrhea in February 2001 in Morocco. Assigned to Moanda, she was admitted to the medical center of this city on 03/07/2001 for bleeding without deterioration of the general condition. The gynecological examination found a non-painful open centered, long-necked uterus leaving a trickle of blood. The beta HCG assay shows a value of 1800 I. U. pelvic ultrasound shows a pregnancy stopped in the process of expulsion presenting a so-called cluster of grapes appearance. Moanda's doctor sends him to the gynecologist at the neighboring regional hospital for appropriate treatment. Given the abundance of metrorrhagia, a sensitive uterus, uterine revision will be performed followed by administration of oxytocic. An ultrasound check-up shows an empty uterus three days later. However, a new beta HCG dosage indicates a level of 3350 I. U. The doctor, convinced that she is dealing with a molar pregnancy, transfers the patient to the center hospitalier de Libreville. The persistence of hemorrhages and the diagnosis mentioned upstream led to the initiation of a treatment consisting of methotrexate by injection, oral methylergometrine, clavulanic acid and oral amoxicillin. Despite this, the persistence of metrorrhagia means that a new curettage is scheduled, this time with a request for anatomy-pathological analysis. A new beta HCG test carried out a week later gave a result of 520 I. U. The downward trend was confirmed on 04/22/2001 with figures standing at 25 I. U. On the ultrasound level, there is no uterine abnormality. Anatomo-pathological examination of the curettage product, for its part, showed young placental villi mummified in a nesting zone rich in migrating intermediate trophoblast cells. Traumatized by the evoked diagnosis of molar pregnancy, the couple on the occasion of the delivery of the histopathological results, had asked all kinds of questions surely reflecting their distress. Finally, on 02/22/2002 we received in the anatomy pathology department this same couple who were extremely kind to present us a newborn of 2 weeks in perfect health.

CONCLUSION

The histopathological study of the endometrium is of great support in Africa, as it contributes to the diagnosis of endometrial bleeding. Complex glandular hyperplasia is an entity requiring morphological and therapeutic monitoring. Its discovery should lead to research into exogenous or endogenous estrogen production. In fact, a climate of hyper-eostrogeny can lead to the development of glandular endometrial carcinoma.

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REFERENCES

1. Lansac J, Lecomte P, Marret H. Gynécologie pour le praticien. SIMEP. Paris. 1994;4.
2. Dallenbach-Hellweg G. Changes in the endometrium caused by endogenous hormonal dysfunction. Verh Dtsch Ges Pathol. 1997;81: 213-8.

3. Hermanek P, Hutter RVP, Sobin LH, Wagner G, Wittekind C. Atlas TNM : Guide illustré de la Classification TNM / pTNM des tumeurs malignes. 4th ed. Springer, editor. France: Broché; 1998.
4. Rodríguez-Manzaneque JC, Graubert M, Iruela-Arispe ML. Endothelial cell dysfunction following prolonged activation of progesterone receptor. Hum Reprod. 2000;15(3):39-47.
5. Clark TJ, Neelakantan D, Gupta JK. The management of endometrial hyperplasia: an evaluation of current practice. Eur J Obstet Gynecol Reprod Biol. 2006;125:259-64.
6. Sakande B, Lankoande J, Ouattara T, Ouedraogo A, Ouedraogo C, Kone B et al. Les biopsies de l endomètre au Centre Hospitalier National Yalgado Ouedraogo De Ouedraogo (Burkina-Fasso). Principales indications et aspects histologique. Med Afr Noire. 1998;45:117-21.
7. Mbakop A, Kassia JM, Nkegoum B. Contribution à l'étude anatomo-clinique des hyperplasie de l endomètre. A propos de 67 cas observés à l'hôpital Central et a l'hôpital Général de Yaoundé. Le J Cameroun méd. 1996;2:20-3.
8. Woodruff JD, Parmley TH, Kurman RJ. Atlas of gynecologic pathology. Raven Press. 1988.
9. Philippe E, Charpin C. Pathologie gynécologique et obstétricale. Masson E, editor. Paris, Masson. 1992.
10. Nuovo MA, Nuovo GJ, McCaffrey RM, Levine RU, Barron B, Winkler B. Endometrial polyps in postmenopausal patients receiving tamoxifen. Int J Gynecol Pathol Off J Int Soc Gynecol Pathol. 1989;8:12531.
11. Delarue T, Baty A, Foissey A. Ménorragies, métrorragies et endomètre: à propos de 300 cas. J gynécologie Obs Biol la Reprod. 1988;17:57-64.
12. Jacks TH, Obed JY, Agida ET, Petrova GV. Dysmenorrhoea and menstrual abnormalities among postmenarcheal secondary school girls in Maiduguri Nigeria. Afr J Med Med Sci. 2005;34:87-9.
13. Kazadi Buanga J. Metrorragies endo-uterines: données etiologiques par l'histologie une série retrospective de 214 curetages. Med Afr Noire. 2001;48:30-2.
14. Kazadi-Buanga J, Jurado-Chacon M. Etiologic study of 275 cases of endo-uterine hemorrhage by uterine curettage. Rev Fr Gynecol Obstet. 1994;89:129-33.
15. Hertig AT. Tumors of the Female Sex Organs: Tumors of the Vulva, Vagina and Uterus. Supplement. Armed Forces Institute Pathol.1968;283-334.
16. Mandengou JR, Minko-Etoua D, Mabika B. Étude rétrospective des hyperplasies de l' endomètre au Gabon. Université des Sciences de la Santé. 2000.
17. Garuti G, Cellani F, Garzia D, Colonnelli M, Luerti M. Accuracy of hysteroscopic diagnosis of endometrial hyperplasia: a retrospective study of 323 patients. J Minim Invasive Gynecol. 2005;12: 247-53.
18. Montgomery BE, Daum GS, Dunton CJ. Endometrial hyperplasia: a review. Obstet Gynecol Surv. 2004;59:368-78.
19. Ferenczy A, Gelfand MM, Tzipris F. The cytodynamics of endometrial hyperplasia and carcinoma. A review. Ann Pathol. 1983;3:189-201.
20. Vakiani M, Vavilis D, Agorastos T, Stamatopoulos P, Assimaki A, Bontis J. Histopathological findings of the endometrium in patients with dysfunctional uterine bleeding. Clin Exp Obstet Gynecol. 1996;23: 236-9.
21. Philippe E, Charpin C. Pathologie Gynécologique et obstétricale. Masson E, editor. Paris. 1993.
22. Meye JF, Mabika, Mabika B, Belembaogo E, Minko Mi Etoua DIN, Engonga Beka T et al. Les carcinomes de l endomètre au Gabon, étude de 34 cas sur 11 ans : 1988-1998. Cah santé. 2000;10:43-5.
23. Schnatz PF, Guile M, O'Sullivan DM, Sorosky JI. Clinical significance of atypical glandular cells on cervical cytology. Obstet Gynecol. 2006;107:701-8.
24. Simionescu C, Florescu M, Niculescu M, Bălă S, Manea M. Histopathologic aspects of the limited endometrial hyperplasias--a study concerning 149 cases. Rom J Morphol Embryol Rev Roum Morphol Embryol. 2005;46:51-5.
25. Bergeron C. Les prélèvements cytologiques et histologiques de l'endomètre pendant la ménopause: Imagerie gynécologique à la ménopause. Reprod Hum Horm. 1997;10:482-5.
26. De Meuron A, De Grandi P, Ruzicka J, Gloor E. Early diagnosis of premalignant and malignant lesions of the endometrium. Evaluation of a device for collecting intrauterine samples (the Inocurette) compared with exploratory curettage. J Gynecol Obstet Biol Reprod (Paris). 1986;15:1035-42.
27. Agostini A, Schaeffer V, Cravello L, Bretelle F, Roger V, Blanc B. Place de l'hystéroscopie dans le diagnostic et la prise en charge des hyperplasies atypiques de l'endomètre. Gynécologie Obs Fertil. 2003;31:355-8.
28. Chamlian DL, Taylor HB. Endometrial hyperplasia in young women. Obstet Gynecol. 1970;36: 659-66.
29. Tabata T, Yamawaki T, Yabana T, Ida M, Nishimura K, Nose Y. Natural history of endometrial hyperplasia. Study of 77 patients. Arch Gynecol Obstet. 2001;265:85-8.
30. Greenwood SM, Wright DJ. Evaluation of the office endometrial biopsy in the detection of endometrial carcinoma and atypical hyperplasia. Cancer. 1979;43:1474-8.
31. Hendrickson MR, Kempson RL. Surgical pathology of the uterine corpus. Major Probl Pathol. 1979;12:1-580.
32. De Brux J. Histopathologie gynécologique. Masson, Editeurs. 1971.

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