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Assessment of cervical cytological atypia among oral hormonal contraceptive pills users in Khartoum Teaching Hospital

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DEDICATION

TO MY PARENTS,

TEACHERS,

AND FRIENDS

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ABBREVIATIONS

OC	Oral contraceptive
Pap.	Papanicolaou.
HPV	Human papillomavirus.
CIN	Cervical intraepithelial neoplasia
HIV	Human immunodeficiency virus.
NCI	National cancer institute
TBS	The Bethesda system
LSIL	Low grade squamous intraepithelial lesion.
HSIL	High grade squamous intraepithelial lesion.
BSCC	British society for clinical cytology.
ASCUS	Atypical squamous cells of undetermined significance.
OG 6	Orange G 6.
EA 50	Eosin azure 50.
ER	Estrogen.
PR	Progesteron.
MGG	May Grunwald Giemsa.
SPSS	Statistical programe for social sciences.
D.P.X.	Desterine plastisizer and Xylene

Abstract

This is a retrospective cohort case control study, conducted in Khartoum Teaching Hospital (Fath El Rahman Elbashir center) during the period from May 2004-Oct 2005 in order to assess the cervical cytological atypia and its degrees among OC pills users by the use of cytological methods.

The study assessed the cytological changes in the cervix among 100 subjects. Of whom, 50 were Oral Contraceptive (OC) pills users (cases), and 50 subjects were non-OC pills users (controls).

Analysis of the cytological smears identified atypia among 9(9%) subjects, and no atypia among the remaining 91(91%) subjects. Of the 9 atypical changes, 7(78%) were detected among cases and only 2(22%) among controls. These findings indicating that OC pills use is a risk factor for cervical cancer, and this was found to be statistically significant ($P<0.006$). Of the 7 atypical changes among cases, 5(71%) showed mild dyskaryosis and the remaining 2(29%) showed moderate dyskaryosis. The 2 atypical smears among control group were found with mild dyskaryosis.

Although, atypical changes appeared after short periods of OC pills use (>1year), the risk for atypia was increasing with the increase of age ($P<0.007$).

Fungal infections were detected among 5(10%) of the study subjects. Of these, 4(80%) were detected among cases and only 1(20%) among controls. These findings indicating that OC pills use increasing the rate of fungal infection, and this was found to be statistically significant ($P<0.001$).

Factors such as duration of OC use, number of child birth should be considered when assessing cervical cytology.

Exfoliated cytology appears to be of value in detection of pre-malignant and malignant lesions. OC pills users should undergo interval cytological screening programes.

ملخص الأطروحة

تم اجراء هذه الدراسة الحالتحكمية فى مشفى الخرطوم التعليمى (مجمع فتح الرحمن البشير) خلال الفترة ما بين مايو 2004 الى اكتوبر 2005 بهدف تحديد التغيرات الخلوية اللانمطية لعنق الرحم وحدتها لدى النساء اللواتى يستخدمن حبوب منع الحمل الهرمونية المركبة ؛ وذلك باستخدام تقنيات علم الخلايا.

تم اجراء الدراسة لمائة امرأة ؛ 50 منهن مستخدمات للحبوب كشريحة مستهدفة والخمسون الأخريات لم يستخدمن الحبوب كمجموعة تحكمية. بتحليل المسحات الخلوية وجدت التغيرات اللانمطية في 9(9%) من مجموعة الدراسة ؛اما باقي المسحات فكانت ضمن المعدل الطبيعي وشكلت 91(91%).

من هذه التغيرات اللانمطية التسعة كانت هنالك 7(78%) في الشريحة المستهدفة و2 (22%) فقط في المجموعة التحكمية.

من هذه الحالات السبعة ضمن الشريحة المستهدفة كانت هنالك 5(71%) حالات سوء خلوي طفيف؛ 2(29%) سوء خلوي متوسط .أما الحالات اللانمطية ضمن الشريحة التحكمية فكانت 2 كلها من النوع الطفيف. وجد أن هذا الاختلاف بين الشريحتين كان اختلافا ذو دلالة احصائية كبيرة ($P<0.006$).

وجد أن التغيرات المذكورة أعلاه تظهر بعد فترة قصيرة (أقل من عام) من استخدام الحبوب كما أن هذه التغيرات تزداد بزيادة العمر ووجد ذلك بدلالة احصائية ($P<0.007$).كما وجدت الاصابات الفطرية بصورة أكبر لدى مستخدمات الحبوب وذلك بدلالة احصائية ($P<0.001$).

ايضا أن عوامل مثل فترة الاستخدام وعدد مرات الحمل الكامل هي عوامل ذات علاقة بالتغيرات الخلوية ويجب اخذها بعين الاعتبار عند اجراء المسح الخلوي لعنق الرحم.

وجدت هذه الدراسة أن تقنية الخلايا المتساقطة هي تقنية ذات قيمة تشخيصية عالية في كشف وتحديد كل من الحالات قبل السرطانية والحالات السرطانية .

أوصت هذه الدراسة بأن مستخدمات حبوب منع الحمل الهرمونية يجب ان يتبعوا نظاما دوريا للكشف الخلوي بهدف تحديد التغيرات الخلوية الغير طبيعية قبل تفاقمها الى حالات سرطانية.

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CHAPTER ONE

INTRODUCTION

Cytology is defined as the study of cells that exfoliated or shed from mucus membranes or obtained by physical means from other parts of the body. Such cells may also be found in body fluids like sputum and peritoneal fluid. Most of the cytological techniques are employed for the rapid and early diagnosis of malignancy particularly in carcinoma of the cervix. Thus, cytological screening detects and treats cervical abnormalities before they develop into cancer. Therefore, it prevents between 80%-90% of cancer cases in women who attends for a smear at least once every five years. For exfoliated cytology no surgical procedure is needed, large areas can be screened and repetition is unlimited (Culling, 1974, Benedet, 1992).

In recent years cytology has gained a great importance due to its role in detection of malignant and premalignant conditions. This, in addition to the ability of cytology to diagnose different types of inflammatory conditions due to parasitic, fungal, bacterial and viral infections, diagnosis of infertility in women by means of hormonal assessment and determination of sex (Poderick, 1992).

Oral contraceptive (OC) pills first become available to women in the early 1960. The convenience, effectiveness and reversibility of action of birth

control pills have made them the most popular form of birth control. However, concerns have been raised about the role that hormone based OCs play in a cervical cancer and how it might contribute to its development (Burkman, 2001).

Worldwide cervical cancer, which usually occurs in the fifth or sixth decade, is a second most common female malignancy in both incidence and mortality (NIH, 1996). About 500.000 women acquire the disease annually. Of these, 75% are from the developing countries. Among whom about 300.000 women die of this disease annually (WHO, 1996). Invasive cervical cancer is uncommon for example in the United States (USA) in comparison to the third world countries. This is due to the effectiveness of screening programs that assess cervical cytology by Papanicolaou (Pap) procedure (Wingo, et al. 1995).

The Pap. procedure was introduced into clinical practice more than 50 years ago and has resulted in a significant reduction in cervical mortality rates (Ronowicz, et al. 1999). This reduction is due to the high specificity of Pap. procedure, which may reach up to 99% (Fahey, et al. 1995).

Oral contraceptive pills are the most widely distributed form of birth control in Khartoum State. There are many studies around the world link between long-term use of hormonal contraceptive pills and cervical cancer. So far, no studies on this subject were carried in the Sudan, where cancer has now become in the top of the 10 major killed diseases. However, breast

cancer is the leading cancer of all female cancers followed by cervical cancer (14.3%) (WHO, 2003).

Therefore, the aim of this study is to assess the effects of hormonal contraceptive pills on cervical epithelial cytopathology, by using cytological techniques.

CHAPTER TWO

REVIEW OF LITERATURE

2.1. Scientific background:

The female genital tract consists of the external genitalia or vulva, vagina and uterus which is composed of cervix, body, paired fallopian tubes and ovaries. The cervix is the lower portion of the uterus, which protrudes into the vagina. The cervix linked to the body of the uterus by the narrow endocervical canal. Cervix is covered by stratified squamous epithelium continuous with that of the vagina. The lumen of which is connected to the uterine cavity by original or 'native' epithelium. The endocervical canal is lined by a single layer of columnar epithelium. The junction between the two types of epithelium is known as squamocolumnar junction.

The endocervical columnar epithelium merges gradually with the endometrial epithelium. These columnar cells with basal nuclei are of regular sizes and shapes with mucoid cytoplasm. Furthermore, some ciliated cells may be seen. The exact location of the squamocolumnar junction varies, before puberty it is generally within the endocervical canal. As the result of hormonal influences at menarche, the cervix increases in volume, which expose the columnar epithelium lining the endocervical canal at its distal end to the hostile vaginal environment. This stimulates the epithelium to undergo squamous metaplasia. This is a normal physiological process and should not be regarded as a precursor to malignant Change.

2.2. Normal appearance of female genital tract cytology:

The original squamous epithelium covers the vagina and is continuous with that of the cervix. This is a stratified and non-keratinizing squamous epithelium, which is separated from the underlying stroma by a basal lamina. The cells mature as they progress towards the surface of the epithelium. The basal layer is adjacent to the basal lamina and the intermediate layer above is defined by the characteristic basket-weave pattern. As the cells mature they become larger with increasing amounts of cytoplasm, until the superficial layer is reached.

Parabasal squamous cells have a rounded shape with well-defined cell borders. The cytoplasm appears thick, homogenous and stained blue/green with Pap. stain. The nucleus which occupies 80-90% of the total cell size and diameter of 15-30 Mm darkly stained with a coarse chromatin pattern.

Intermediate squamous cells are polygonal with basophilic staining cytoplasm. There may be a yellow-staining perinuclear region, which denotes the presence of glycogen. The nucleus is round with a fine chromatin pattern.

Superficial squamous cells are polygonal cells with angular borders measure 45-50 Mm in diameter with pale pink-stained cytoplasm. The nuclei are small pyknotic of less than 5 Mm in diameter. Although they have tendency for keratinisation but this is not a normal feature in the cervix.

Endocervical glandular cells in a cervical smear are easily recognised. They occur in large groups and small sheets and sometimes whole villi may

be seen. The cells have indistinct borders with finely vaculated pale blue/grey stained cytoplasm. The nuclei have a finely granular chromatin pattern with prominent chromocentres. Cilia are rarely seen, although a terminal plate may be identified. Furthermore, the cells show a honeycomb appearance, and occasionally the cytoplasm may be stained a pink hue.

Endometerial cells are normally found in cervical smears up to the 8th day of the cycle. Hence endometerial cells can be seen up to the 10-12th day. Any presence outside this period should be regarded as an abnormal finding. These cells are seen in tightly packed groups as darkly stained cells with irregular, coarsely clumped chromatin. However, there are some nuclear irregularities, reflecting degenerative cell change. At about the 5-7th day the cells may occur singly or in loosely associated streaks, and may be mistaken for histiocytes (Bancroft, et al. 1996).

2.3. Cytology of cervical intraepithelial neoplasia:

The term Cervical Intraepithelial Neoplasia (CIN) is a single descriptive term for all grades of dysplasia as well as carcinoma in situ (Evan, et al. 1986).

It is well known that squamous cell carcinoma of the cervix is preceded by precancerous changes in cervical epithelium of the transformation zone. This condition is characterized by a replacement of normal cervical squamous epithelium with neoplastic cells showing varying degrees of abnormalities. (Springs, et al. 1978).

Neoplastic cells from the surface of the CIN lesions can be distinguished from the normal cells in the cervical smears by many characteristics. These include the presence of nuclear enlargement results in abnormal nuclear cytoplasmic ratio, chromatin clumping, increase mitotic figure, variation in nuclear size and shape, irregularity of the nuclear outline, hyperchromasia, multi nucleation and large irregular and some times multiple nucleoli. CIN in cytology is known as dyskaryosis. The degree of dyskaryosis may be mild, moderate or severe depending on the previously mentioned characteristics. Mild dyskaryosis reflects the presence of CIN I, moderate dyskaryosis reflects the presence of CIN II and severe dyskaryosis reflects the presence of CIN III (Evan, et al. 1986).

Cytology of CINI: - Mild dyskaryosis characterized by the presence of one or more irregular nuclei which occupy less than one half of the total area of cytoplasm, in addition to increased clumped chromatin. Furthermore, the cells resemble superficial or intermediate squamous cell with slightly enlarged irregular nuclei.

Cytology of CINII: - Neoplastic cells in the smear contain comparatively larger nuclei than those showing mild dyskaryosis. The nuclei occupy one-half to two thirds of the cytoplasmic area. The cell resembles a superficial or intermediate cell with a considerably enlarged nucleus.

Cytology of CINIII: - The smear contains severe dyskaryotic cells with massive nuclear changes. The nuclear cytoplasmic ratio is high and the nuclei occupy more than two thirds of the cell cytoplasmic area. The

cytoplasm of dyskaryotic cell may be very scanty particularly in case of presence of undifferentiated neoplastic cells. If there is some surface maturation or flattening, the amount of cytoplasm will be greater and may be keratinized. Some times dyskaryotic nuclei without cytoplasm may be found in a cytological smears. Hypochromasia, which is termed as pale dyskaryosis frequently observed in smears from lesions arising from atrophic epithelium of old women. Macro nucleoli and/or bizarre-shaped cells are more commonly associated with invasive cancer (Coleman, 1999).

2.4. Epidemiology of cervical cancer:

Although cervical cancer is associated with a broad age, it usually occurs at a mean age of 54 years (Barber, 1982). In contrast, intraepithelial lesions, which are the precursors of invasive disease, frequently occur in younger women (often under 40 years of age) (Cramer, 1997). These precursor lesions, known as cervical intraepithelial neoplasia (CIN) (Richart, 1973).

2.4.1. Aetiology of cervical carcinoma:

Since 1960 and up to early eighties the aetiology of cervical carcinoma was attributed to the cytomegalovirus and later the Herpes simplex type II (WHO, 1995). However, now it is well established that the cervical cancer is related to oncogenic strains of the Human Papilloma Virus (HPV), notably types 16 and 18 (WHO, 1996, CLifford, et al. 2003). Although there are other strains of HPV considered as oncogenic. Furthermore, type 18, which associated with a rapid onset of disease, usually affects the endocervical

glands. Nevertheless, 99.7% of cases of cervical cancer and severe CIN II/III are associated with previous oncogenic infections (Judson, 1992, Walboomers, et al. 1999) However, Morrison, et al. (2001) studied 5 cases of unusual variants of large invasive squamous cells carcinoma of the cervix for detection of HPV. HPV PCR test did not detects HPV DNA in the specimens.

2.4.2. Risk factors for cervical cancer:

HPV is the most sexually transmitted infection in the world. It occurs in up to 75% of sexually active women. Changes to cervical cancer may develop through a wide period of time which may take up to 20 years from the first infection. Only about 10% of infected women would go on to develop cervical dysplasia and of these only a few would develop overt cancer of the cervix (Bosch, et al. 1995, Groopman, 1999). In addition to HPV, there are other factors contribute in carcinomatous changes. These include behavioral, social, cultural and economic factors. Multiple male sexual parteners, male sexual parteners who themselves have had multiple sexual parteners (Christopherson, et al. 1965, Rotkin, 1967, Kessler, 1976). Cigarette smoking has also been implicated as a co-factor (Winkelstein, 1999). Ylitalo, et al. (1999) in a cohort case control study by using cytological screening method confirmed the association between smoking and cervical cancer. It has been suggested that the risk of cervical cancer is also increased in immunosuppressed patients as a consequence of renal-allograft transplantation or Hodgkin's disease (Schneider, 1983, Halpert, et

al. 1986, Katz, 1987, Stenlella, 1998, Mcdonald, 1999). However, the immunosuppression caused by human immunodeficiency virus (HIV) infection was found to be a risk factor for the development of CIN (Maiman, et al. 1990, Maiman, et al. 1993, Klein, et al. 1994). History of cervical carcinoma in close relatives may also be an important factor (Magnusson, et al. 1999).

The role of the combined oral contraceptive pills as a risk factor for cervical carcinoma has been clarified (Schiffman, et al. 1996).

2.5. Cervical Cytological screening:

Cytological screening for premalignant lesions of the cervix has been introduced over 50 years. In the 1940's George Papanicolaou first described five classes of cellular changes in exfoliated cells of the cervix (Papanicolaou, 1963). Evidence of the effectiveness of Pap-smear screening is largely derived from retrospective analysis of the incidence of cervical cancer and associated mortality. In the mid-1960s, Finland, Sweden, and Iceland implemented screening programs in which more than 80 per cent of women participated. Norway performed screening in only one county, which comprised 5 per cent of the population. All four countries noted a similar incidence of cervical cancer. This is not surprising, when concerning the homogeneity of their populations.

However, the incidence of cervical cancer did not change in Norway for more than 20 subsequent years in concerning the other three countries, mortality from cervical cancer decreased by approximately 50%

(Johannesson, et al. 1978). Likewise, with the implementation of Pap-smear screening programs in British Columbia, the incidence of cervical cancer decreased by 85 per cent between 1955 and 1988 (Benedet, et al. 1992). Similarly, mortality from cervical cancer in the United States decreased by 70 per cent between 1947 and 1984 by introduction of mass screening programs (Devesa, et al. 1987, Koss, 1989).

Furthermore, many studies provide reliable evidences for the effectiveness of Pap-smear screening in the prevention of cervical cancer (Worth, 1984, Cook, et al. 1984, Day, 1984).

The efficacy of Pap-smear screening is largely dependent on the quality of the specimen and the accuracy of the cytologic interpretation. Pap smears may be reported as technically inadequate as the result of sampling errors in 12.3 per cent of cases. Thereby, the reported findings may underestimate the intraepithelial lesion in 17.5 percent of cases (Van der Graaf, et al. 1987). Likewise, it has been estimated that approximately 15 to 25 per cent of patients with intraepithelial lesions have normal Pap-smear results (Shingleton, et al. 1995, Niloff, et al. 1995).

Such false negative results can be minimized by ensuring that the proper technique is used to obtain the cytologic specimen. The transformation zone is the most common site for the development of intraepithelial lesions that may give rise to invasive disease. False negative results may be due to inadequate sampling of the transformation zone. Notably, transformation

zone often regresses into the endocervical canal in postmenopausal women (Shingleton, et al. 1995).

The most cost-effective interval for Pap-smear screening is unknown. The American College of Obstetricians and Gynecologists and the American Cancer Society recommend that annual screening commence when women become sexually active or reach age of 18 years (Shingleton, et al. 1995, Niloff, et al. 1995).

2.5.1. Interpretation of Pap-smears:

A definitive diagnosis of CIN or carcinoma can be made only by biopsy of suspicious lesions observed either grossly or during colposcopy. The Pap-smear is a screening test designed to identify patients who may have premalignant or malignant lesions requiring further evaluation (Shingleton, et al. 1995).

Several cytologic classifications of Pap-smear findings have been proposed. In the 1940's George Papancolaou first described five classes of cellular changes in exfoliated cells of the cervix. These include:

- Class 1: Absence of atypical or abnormal cells.
- Class 2: Atypical cytology but no evidence of malignancy.
- Class 3: cytology suggestive but not conclusive.
- Class 4: cytology strongly suggestive of malignancy.
- Class 5: cytology conclusive for malignancy.

This original classification was adopted worldwide and it has been the cornerstone of the screening programs in the Nordic countries since the 1960's (Surjanen, 1995, Hakam, 1997).

In order to correlate the histological findings with the appropriate cytological abnormality, the British Society for Clinical Cytology (BSCC) introduced a new system of classification of the cytological specimens in the 1980's. This system employed the term dyskaryosis, which was further subdivided into mild, moderate and severe. These subdivisions are supposed to correlate very well with the histological grades of CIN I, II and III (Evans, 1986). In 1988 the National Cancer Institute (NCI) of the USA came out with a new system, the Bethesda System (TBS) for reporting cervical and vaginal cytological diagnosis (The Bethesda, 1988, Kurman, 1991). Furthermore, the NCI of USA established another system in 1991. This new system simplified the three systems of mild moderate and severe dyskaryosis to Low Grade Squamous Intra-epithelial lesion (LSIL), High Grade Squamous Intraepithelial Lesion (HSIL) (Herbst, 1992, Sherman, 1992, Tabbar, 1992, Kurman, 1994). Cytological reports are then given as follows:

- Within normal limits.
- Atypical Squamous Cells of Undetermined Significance (ASCUS).
- Cellular changes suggestive of:
- LSIL.
- HSIL.

2.6. Oral contraceptive:

Oral contraceptive (OC) pills are the most effective, reliable and popular form of reversible contraception. The use of OC pills has been growing since 1970. Now, more than 100 million women in the world are using OC pills. Still its use in developing countries is less popular than developed countries (6% vs. 14% married women in reproductive age). However, concerns have been raised about the role that hormones play in a number of cancers, and how hormone-based OCs might contribute to their development (Burkman, 2001).

Oral contraceptive pills work primarily by inhibiting the ovaries from releasing ova (inhibiting ovulation). The cervix also produces thicker mucous so that it is more difficult for sperm to travel. The pills may also act on the endometrium to make implantation more difficult.

Currently, two types of OCs are available. The most commonly prescribed OCs contain two man-made version of natural female hormones (Estrogen (ER) and Progesterone (PR)) that are similar to the hormones the ovaries normally produce. ER stimulates the growth and development of the uterus at puberty, causes the endometrium to thicken during the first half of the menstrual cycle, and causes changes in breast tissue at puberty. PR,

which is produced during the last half of the menstrual cycle, prepares the endometrium to receive the ova. If the ovum is fertilized, progesterone secretion continues, preventing the release of additional ova from the ovaries. For this reason, PR is called the "pregnancy-supporting hormone", and it has valuable contraceptive effects. The man-made PR used in OCs is called progestogene or progestin.

The second type of OCs is called the mini pills. Which contains only a progestogen. The mini pills are less effective in preventing pregnancy than the combinated pills. (Hatcher, et al. 1998).

2.6.1. Risk of oral contraceptive pills:

The risk of ischemic stroke is 1.5 times higher in women with hypertension who are taking OC pills (WHO, 1996).

Women taking OC pills with higher ER doses are at greater risk for ischemic stroke. Hypertension and smoking are independent and additive risk factors for myocardial infarction, ischemic stroke and haemorrhagic stroke in patients taking OC pills (WHO, 1997, Hatcher, et al. 1998). The risk of mortality from cardiovascular disease attributable to OC pills use is up to 10 times higher in women 40 to 44 years of age than in women 20 to 24 years of age (WHO, 1998). Women take OC pills have three to six times greater risk of venous thromboembolism than women who does not use this contraceptive method (Chasen, et al. 1998). The absolute risk of venous thromboembolism associated with OC pills increases with age, obesity,

recent surgery and some forms of thrombophilia. This risk is highest during the first year of use (Carr, et al. 1997).

2.6.2. Oral contraceptive and cancer risk:

A recent meta-analysis of 54 epidemiological studies concluded that there is a small increased risk of breast cancer in recent user of OC pills. The risk falls gradually over the period of ten years of use and after 10 years risk is equivalent to non-users. Additionally, breast cancer developing in OCs users is more localized and has better prognosis (Collaborative group study, 1996). There is a strong association between OCs use and hepatocellular adenoma. This hormonally responsive tumour can cause fatal haemorrhage. It usually regresses after discontinuation of drug and risk is related to duration of use (Rooks, et al. 1979).

2.6.3. Oral contraceptive and cervical cancer:

In USA approximately 13,000 women had the new diagnosis of cervical cancer in 1997. This is observed more frequently in contraceptive pills users than in non-users. This results in an increased risk ratio of about 1.5-1.9. An uncommon form of cervical cancer and adenocarcinoma also shows an increased risk ratio of about 2.0-2.5 with pill using (Kaunits, et al. 1998). However, there are some evidences that long-term use of OCs may increase the risk of cancer of the cervix as indicated by a meta analysis based on data from 28 studies (Smith, 2003). Other studies found that pills use in women less than 20 years old means 280% higher risk of cervical

cancer. In women 20-24 years, it is 70% higher, and in women 25-29 years it is 40% higher (Kohler, et al. 1994).

Another study cites increased risk of 250% for cervical cancer amongst pill users (Ursin, et al. 1994). Long term users (6-12 years) have increase risk of 100-340% to develop cervical cancer than non users (Brisson, et al. 1994, Kohler, et al. 1994). However, Chen, et al. (1996) reported that women who used the pills for only 1-6 months had a 190% increase in cervical cancer than non-users.

2.6.4. Oral contraceptive and cervical atypia:

There are many studies link between OCs and cytological changes and atypia in cervical smears.

Pescetto, et al. (1974) in Italy studied the effects of estrogen-progestin combinations on vaginal cytology by using colpocytological smears from 598 women aged 18-45 years who had used OCs for various durations from 3 months to 6 years. In 8% of cases, serious morphological abnormalities characterized by dyskaryosis were observed.

In France, De Brux (1974) investigated the incidence of cervical lesions in 11, 090 women using high-dose estrogen-progestagens, 11,376 using low-dose pills. Among the high-dose group there were 140 (1.27%) atypical smears with 100 slight dyskaryosis, 21 severe dysplasias, 5 hyperplasias, 12 carcinomas in situ, and 2 invasive carcinomas. The low-dose group had 59 (0.51%) atypical results, with 45 slight dyskaryosis, 7 severe dysplasias, 2 hyperplasias, and 5 carcinomas in situ.

Livingston (1976) found an unusually high increase in the incidence of suspicious Papanicolaou smears among women 15-22 years old. Cervical tissue from these patients has shown a different form of atypia than usually encountered. Both dyskaryosis and karyomegaly have been observed. Follow-up study revealed that the majority of the patients were sexually promiscuous and/or using birth control pills.

Histological changes in cervical epithelium under influence of OCs were studied by El Hakim, et al. (1976) in 100 patients used different types of combined OCs, 50 non-OCs users served as controls. The ectocervical epithelium revealed increased vascularity and its penetration by perpendicular vascular channels reaching two-thirds of its thickness in 65% of cases. Basal cell hyperplasia was increased in 38% of patients. Vesicles between layers of squamous epithelium were observed in 26% of users as compared to 3% of controls. A single case of mild dysplasia was detected. Vascularity of the endocervical stroma was increased in 83% of users compared to 30% of controls. Seventy nine per cent of OC users showed endocervical glandular hyperplasia, the most prominent finding, compared with 22% of controls.

In France, Favre, et al. (1979) noted that OCs could cause a number of morphological disorders in the cervico-vaginal cytology. They investigated cytological smears from 1685 patients on OC pills and compared them to 1000 smears from women not on OC. Dyskaryosis was found in 3.6% of OCs patients, and 1.4% of controls.

A comparative retrospective study of cervical lesions was conducted by Arizago (1979) among 154,784 women. Of these, 36.7% of women observed used hormonal contraception. Dysplasias was found in 1.71/1000 women, but in 1.2/1000 of those using contraception. Incidence of carcinoma in situ was 1.97/1000 over all, but 1.84/1000 for women on contraception. Patients who used contraception, and who had either dysplasia or carcinoma, tended to be younger than patients who did not use contraception; the difference was 5 years for dysplasia, 10 for carcinoma in situ and 16 for invasive carcinoma.

Kwikkel, et al. (1985) reported on the variation in nuclear and cytoplasmic size of intermediate cells in normal cervical smears, in relationship to the week of the menstrual cycle and in relationship to the mode of contraception. A total of 18000 cells from 360 different women were studied. A significant difference in nuclear size of cells in smears from ovulating women not using contraception was found in comparison with intermediate cells in smears from women using contraceptive pills ($P < 0.02$).

In Croatia, Ljuca, et al. (2000) examined a relationship between OCs use and precancerous and early cancerous lesions of the cervix by using Pap. test. Results have shown that there is high significant positive relationship between oral contraceptive use and precancerous and early cancerous lesions of the cervix. The users of OCs have shown Pap. III and Pap. IV smears grades 5-10 years earlier than non-users.

Morrison, et al. (2003) noted a significant increase in the diagnosis of (ASCUS) in Thin Prepared Pap. smears from premenopausal women using oral contraceptive pills (9%) versus women not use OCs (4%) (P=0.02).

In Egypt, Darwish, et al. (2004) performed clinical and cytologic evaluations of the cervix for 200 patients using OCs and 125 patients wearing Intrauterine Devices. Initial naked eye assessment of the cervix revealed statistically significant difference between both groups ($p < 0.000$). However, cytologic examinations revealed positive cases of low and high-grade squamous intraepithelial lesion (SIL) in 38(19%) and 22(17.6%) among users and non-users respectively.

2.7. Cervical cytological techniques:

2.7.1. Collection of cervical smears:

For most cytology laboratories the greatest proportion of specimen consists of cervical smears. The most widely acceptable method of obtaining a satisfactory sample is by the use of a modified Ayres spatula. The optimum sample should provide evidence that the transformation zone has been covered and that the squamocolumnar junction has been reached. (Wolfendal, 1989). The spatula has an elongated prong to facilitate sampling within the external os of the endocervical canal. The broad blade is capable of sampling a wide area of the ectocervix, covering the transformation zone. The cervix must be completely visualised after the introduction of a cervical speculum. Lubricant is best avoided, since contamination of the smear by lubricant jelly may render it unsatisfactory.

The use of a cotton wool swab or endocervical brush alone is not advocated for routine cervical smears, as only part of the cervix will be sampled. All scraped materials are spread directly onto glass slide and fixed immediately in 95 per cent ethanol.

2.7.2. Fixation of cervical smears:

Most cervical smears are stained by the papanicolaou method. In order to preserve cell details without distortion, the smears must be wet-fixed rapidly before the occurrences of any air drying. Papanicolaou`s original fixative of equal parts of 95 per cent ethyl alcohol and ether has been largely abandoned because of the fire hazards associated with ether. Most laboratories now used 95 per cent ethyl alcohol. Additionally, coating fixatives are widely used for cervical smears. The smear is covered in a waxy coating, which protects it from damage. Most of these fixatives are composed of polyethylene glycol in an alcoholic base and are applied either from a dropper bottle or in spray form.

2.7.3. Staining of cervical smears:

Consistency and reliability in staining are the cornerstones of cytological interpretation. Subtle cell appearances are constantly being compared and assessed during screening. However, in most instances cytologists rely heavily on the quality and appearance of the stain. The universal stain for cytological preparations is the Papanicolaou stain. Harris’s haematoxylin is the optimum nuclear stain and the combination of Orange G 6 (OG 6) and

Eosin Azure 50 (EA 50) give the subtle range of green, blue and pink hues to the cell cytoplasm (Bancroft, et al. 1996).

The principle of reaction can be regarded as an acid base reaction. The cytoplasmic staining may be influnced by the thickness and fixation of the smear. However, Pap. stain provides good differential staining and as result it used widely for other routine cytological smears (Walter, et al. 1996).

Romanowesky stains such as May Grunwald Giemsa (MGG) used to stain air-dried smears. These stains consist of methylene blue/azure B and eosin, dissolved in acetone-free methanol. These stains include Jenner, Giemsa, MGG, and Leishman stains. The pH of the staining solution is critical and ideally should be adjusted. More acid pH levels give more selective chromatin staining and less cytoplasmic basophilia. Less acidic acid pH levels give denser nuclei and increased cytoplasmic basophilia. The pH range should be between 6.4 and 6.9 (Carleton, 1980, Walter, et al. 1996, David, 2000)

CHAPTER THREE

STUDY OBJECTIVES

3.1. The aims of this study were to:

- Assess the cervical cytological atypia (if any) following the use of oral contraceptive pills.
- Determine the relationship between the duration of hormonal contraceptive pills use and risk for cervical premalignant and malignant changes.

CHAPTER FOUR

MATERIALS & METHODS

4.1. Study design:

This is a retrospective cohort case control study to quantify the risk of cervical cytological atypia induced by OC pills use. The study was conducted in Khartoum Teaching Hospital (Fath El Rahman El Basher centre) in Khartoum state during the period from May 2004 – Oct 2005.

4.2. Study population:

One hundred subjects were selected for this study. Fifty subjects were either currently hormonal contraceptive pills users or quit the use not more than 3 months (cases). Fifty subjects were non-hormonal contraceptive pills users (controls). All study subjects were selected by random selection method. Alcoholic, Tobacco users and non-married women were excluded, both from cases and controls.

4.3. Material:

Cytological materials obtained from the cervix were used in this study.

4.4. Sample collection:

One hundred cervical smears were collected by the consultant gynecologist. The cervix was visualized by passing a speculum into the vagina, and cervical scrape was obtained with standard plastic Szalay-cytospatula. The tongue of the spatula was introduced into the endocervical

canal, whilst its shoulder was positioned on 3 o'clock position of the ectocervix. This spatula was then rotated in a clockwise direction through 360^0. The obtained materials were smeared onto cleaned standard microscopical slide and immediately fixed while it was wet in 95% alcohol for 15 min. Then the smears were sent to the laboratory for further processing.

4.5. Sample processing:

All of the smears were processed adopting Pap. Procedure as described by Bancroft, et al. (1996).

The alcohol fixed smears were hydrated in descending alcohol concentrations of 95% through 70% to distilled water for 2 mins in each stage. For staining of nuclei the smears were treated with Harris`s haematoxylin for 7 mins, then it were rinsed in distilled water and differentiated in 0.5% aqueous hydrochloric acid for 3 seconds to remove the excess stain particles, and immediately rinsed in distilled water to stop the action of decoloration. Then, the smears were blued in alkaline water (0.1% aqueous ammonia) for 4 sec., and then dehydrated in ascending alcohol concentrations from 70% through two changes of 95% alcohol 2 mins for each change.

For the cytoplasmic staining, smears were treated with OG 6 for 2 mins, rinsed in 95% alcohol, then treated in EA 50 for 3 mins. Finally smears were hydrated in absolute alcohol, cleared in xylene and mounted in Disterene Plastisizer and Xylene (D.P.X).

Results under light microscope were as follow:

The nuclei……. blue.

Cytoplasm (non keratinising squamous cells)… blue or green.

Keratinizing cells… pink/orange.

4.6. Data recording and analysis:

The atypia included dyskaryosis were assessed cytologically. The presence of two or more of the following features indicating cytological atypia:

Nuclear enlargement associated with increased nuclear cytoplasmic ratio, hyperchromasia, chromatin clumping with moderately prominent nucleolation and irregular nuclear border, bi or multinucleation, increased keratinization and scantiness of the cytoplasm and variation in size and/or shape of the cells and nuclei.

All smears were screened under 10x followed by 40x and the results were reported by using BSCC system. In this system, neoplastic changes were reported in three grades according to its severity. Mild dyskaryosis corresponded to LSIL, while moderate and severe dyskaryosis corresponded to HSIL in Bethesda system. All the smears have fair staining quality. All quality control measures were adopted during the study. All smears were examined by 3 independent investigators, and then, their results were compared and found to be matched in all cases of atypia. All results and information obtained previously in the questionnaire were

prepared in a master sheet, then entered a computer for analysis using SPSS program.

4.7. Ethical consideration:

Each individual in this study was told about the study before taking the specimen. All samples were obtained ethically by the use of disposable spatula.

CHAPTER FIVE

RESULTS

In This retrospective cohort case control study, cervical cytological atypia were assessed among 100 women. Of whom 50 were OC pills users (cases) and 50 were non-OC pills users (controls). Their age ranging between 19-50, with a mean age of 35 years old.

Most of the study subjects were found among age group 31-40 constituting 43(43%) followed by 21-30, 41-50 and <20 constituting 31(31%), 24(24%), 2(2%) respectively, as shown in **Table. 1., Figure. 1.**

Table. 2., Figure. 2. Showing the distribution of study population by occupation. The majority of the study subjects were House-Wives (H/W) constituting 79(79%) followed by teachers 8(8%), workers 5(5%), accountants 2(2%), employee 1(1%), vegetable seller 1(1%), doctor 1(1%), nurse 1(1%), dentist 1(1%) and laboratory assistant 1(1%).

Atypias were detected among 9(9%) of the study subjects. Of these 9 atypical changes, 7(78%) were detected among cases compared to only 2(22%) among controls. Therefore, use of OC pills is a risk factor for occurrence of cervical cytological atypia and this was found to be statistically significant (P< 0.006) as shown in **Table. 3., Figure. 3.**

Concerning the degree of dyskaryosis, of the 7 atypical changes among cases, moderate dyskaryosis were detected in 2(29%) cases, and the remaining 5(71%) were with mild dyskaryosis. Notably, the 2 atypical changes among controls were found with mild dyskaryosis as indicated in **Table. 4., Figure. 4., Photos. 26, 27, 28, 29, 30, 31.**

Table. 5., Figure. 5. Showing the distribution of study population by age and cytological atypia. However, the cytological atypias were found to increase with the increase of age, and this was found to be statistically significant (P<0.007). However, age ranges 31-40 and 41-50 showed the highest numbers of atypia 4(44.5%) for each, followed by 21-30 constituted 1(11%).

Table. 6., Figure. 6. Showing the description of cytological atypia by duration of OC pills use. Duration between 1-5 years showed the highest cytological atypia constituted 4(57%), followed by <1 year, which constituted 3(43%). No cytological atypia was detected in durations between 6-10 and >10 years.

Table. 7., Figure. 7., Photos. 23. Showing the description of study population by exposure and bacterial infection. Bacterial infections were

detected among 10(10%) of the study subjects. The number of positive cases were equal in both case and control groups, constituted 5(50%) for each.

Fungal infection was detected among 5(5%) of the study subjects, of these 5 cases, 4(80%) were detected among case group and 1(20%) was detected among control group as shown in **Table. 8., Figure. 8., Photos. 24, 25.**

Table. 9., Figure. 9. Showing the description of study population by atypia and frequency of childbirths. Atypia was detected among 9 of the study subjects. Frequency of childbirth between 5-8 and <4 showed the highest cytological atypia constituted 4(44.5%) for each, followed by >9 which constituted 1(11%).

Table. 10., Figure. 10. Showing the distribution of study population by lactation and atypia. Most of study subjects were non-lactated. However, atypia were detected in 8(89%) non-lactated, compared to 1(11%) of the lactated women.

Table. 11., Figure. 11. Showing the description of atypical colposcopic cervical features by exposure. There was no difference in atypical cervical features among both case and control groups.

Table 12. , Figure. 12. Showing the description of cervical features by exposure. In case group 24(48%) were found with normal feature, 18(36%) ectopy, 5(10%) metaplasia, 1(2%) napothian follicle, 1(2%) cervical polyp and 1(2%) cervecitis, compared to 24(48%) with normal feature, 17(34%)

ectopy, 3(6%) metaplasia, 3(6%) cervical polyp, 1(2%) napothian follicle, 1(2%) cervecitis and 1(2%) metaplasia&napothian follicle among control group.

Table (1): Distribution of study population by age.

Age(in years)	Frequency	Percentage	Cumulative
< 20	02	02%	02
21 – 30	31	31%	33
31 –	43	43%	76

40			
41 – 50	24	24%	100
Total	**100**	**100%**	

Table (2): Distribution of study population by occupation.

Occupation	**Users**		**Non-Users**		**Total**
	No	**%**	**No**	**%**	
H / W*	38	76%	41	82%	**79**
Accountant	1	2%	1	2%	**2**
Employee	1	2%	0	0%	**1**
Worker	4	8%	1	2%	**5**
Vegetable	1	2%	0	0%	**1**

seller					
Teacher	4	8%	4	8%	**8**
Doctor	1	2%	0	0%	**1**
Nurse	0	0%	1	2%	**1**
Dentist	0	0%	1	2%	**1**
Lab. Assistant	0	0%	1	2%	**1**
Total	**50**	**100 %**	**50**	**100%**	**100**

H / W*: House wife

Table (3): Description of cytological atypia by exposure.

Exposure	**Normal**		**Atypia**		**Total**
	No	**%**	**No**	**%**	
Users	43	47%	7	78%	**50**
Non-users	48	53%	2	22%	**50**
Total	**91**		**9**		**100**

		100%		**100%**	

(P < 0.006)

Table (4): Description of the degree of cytological atypia by exposure.

Atypia	**Users**		**Non-users**		**Total**
	No	**%**	**No**	**%**	
Mild dyskaryosis	5	71%	2	100%	**7**
Moderate dyskarsosis	2	29%	0	0%	**2**
Severe dyskaryosis	0	0%	0	0%	**0**
Total	**7**	**100%**	**2**		**9**

				100%	

Table (5): Description of atypia by age.

Age(in years)	**Normal**		**Atypia**		**Total**
	No	**%**	**No**	**%**	
< 20	2	2%	0	0%	2
21 – 30	30	33%	1	11 %	31
31 – 40	39	43%	4	44. %	43
41 – 50	20	22%	4	44. %	24
Total	**91**	**100%**	**9**	**100 %**	**100**

(P < 0.007)

Table (6): Description of cytological atypia by duration of OC pills use.

Duration (in years)	**Normal**		**Atypia**		**Total**
	No	%	**No**	**%**	
< 1	18	42%	3	43 %	21
1 – 5	21	49%	4	57 %	25
6 – 10	03	7%	0	05 %	03
10+	01	2%	0	0%	01
Total	**43**	**100 %**	**7**	**100 %**	**50**

Table (7): Description of study population by bacterial infection.

Exposure	Positive		Negative		Total
	No	%	No	%	
Users	05	50%	45	50%	50
Non-users	05	50%	45	50%	50
Total	**10**	**100%**	**90**	**100%**	**100**

Table (8): Description of study population by fungal infection.

Exposure	Positive		Negative		Total
	No	%	No	%	
Users	4	80 %	46	48 %	50
Non users	1	20 %	49	52 %	50
Total	**5**	**100**	**95**	**100**	**100**

(P < 0.001)

Table (9): Description of cytological atypia by the frequency of childbirth.

Number	Normal	Atypia	Total

of childre n	No	%	No	%	
< 4	70	77%	4	44.5%	74
5 – 8	19	21%	4	44.5%	23
> 9	2	2%	1	11%	3
Total	**91**	**100%**	**9**	**100%**	**100**

Table (10): Description of cytological atypia by lactation.

Lactation	Normal	Atypia	Total
Lactated	6	1	7

Non-lactated	85	8	93
Total	**91**	**9**	**100**

Table (11): Description of atypical cervical features by exposure.

Exposure	**Normal**		**Atypical cervical features**		**Total**
	No	**%**	**No**	**%**	

Users	24	50%	26	50%	50
Non-users	24	50%	26	50%	50
Total	**48**	**100%**	**52**	**100%**	**100**

Table (12): Description of cervical features by exposure.

Cervical features	Users	Non users	Total
Normal	24	24	**48**
Ectopy	18	17	**35**

Metaplasia	5	3	**8**
Napothian follicle	1	1	**2**
Cervical polyp	1	3	**4**
Cervecitis	1	1	**2**
Metaplasia&Napothian follicle	0	1	**1**
Total	**50**	**50**	**100**

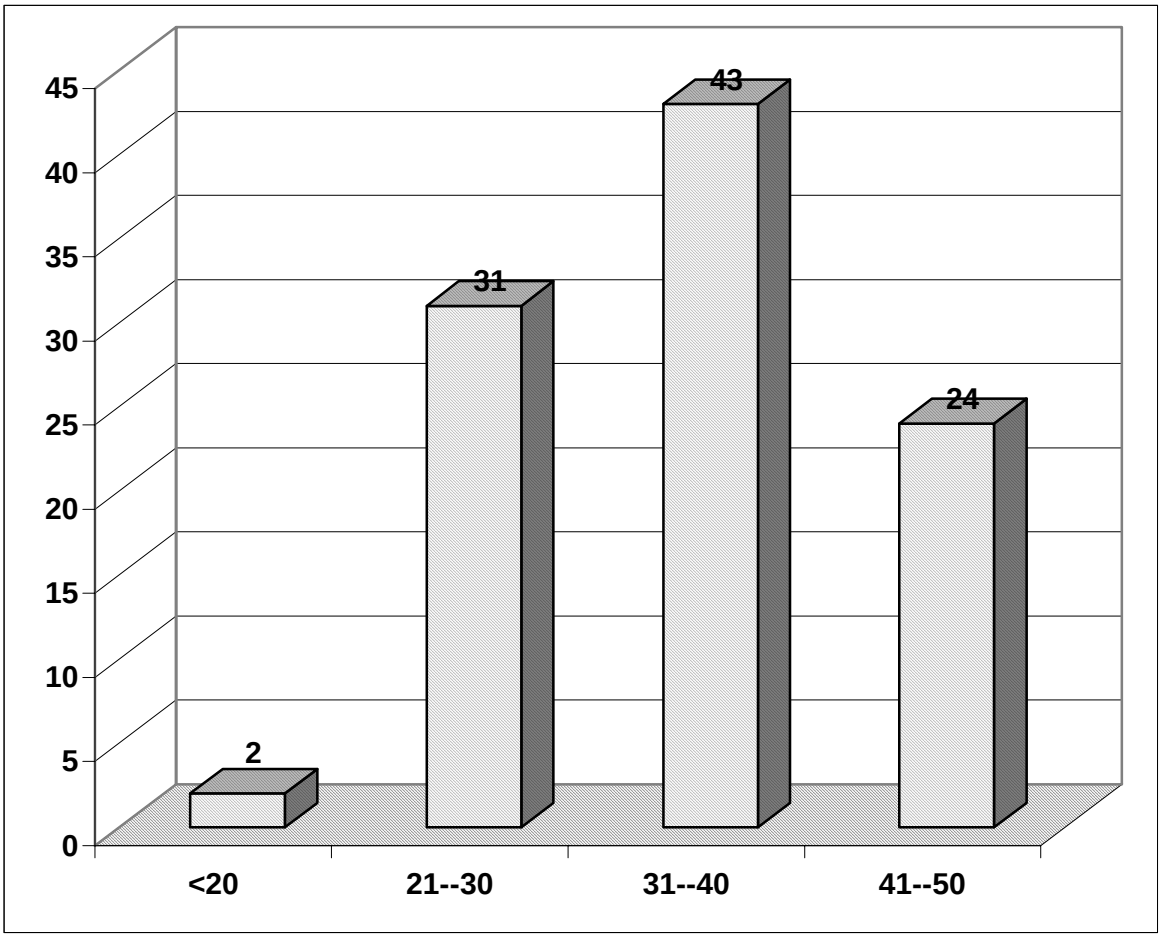

Figure 1: Distribution of study population by age (in years).

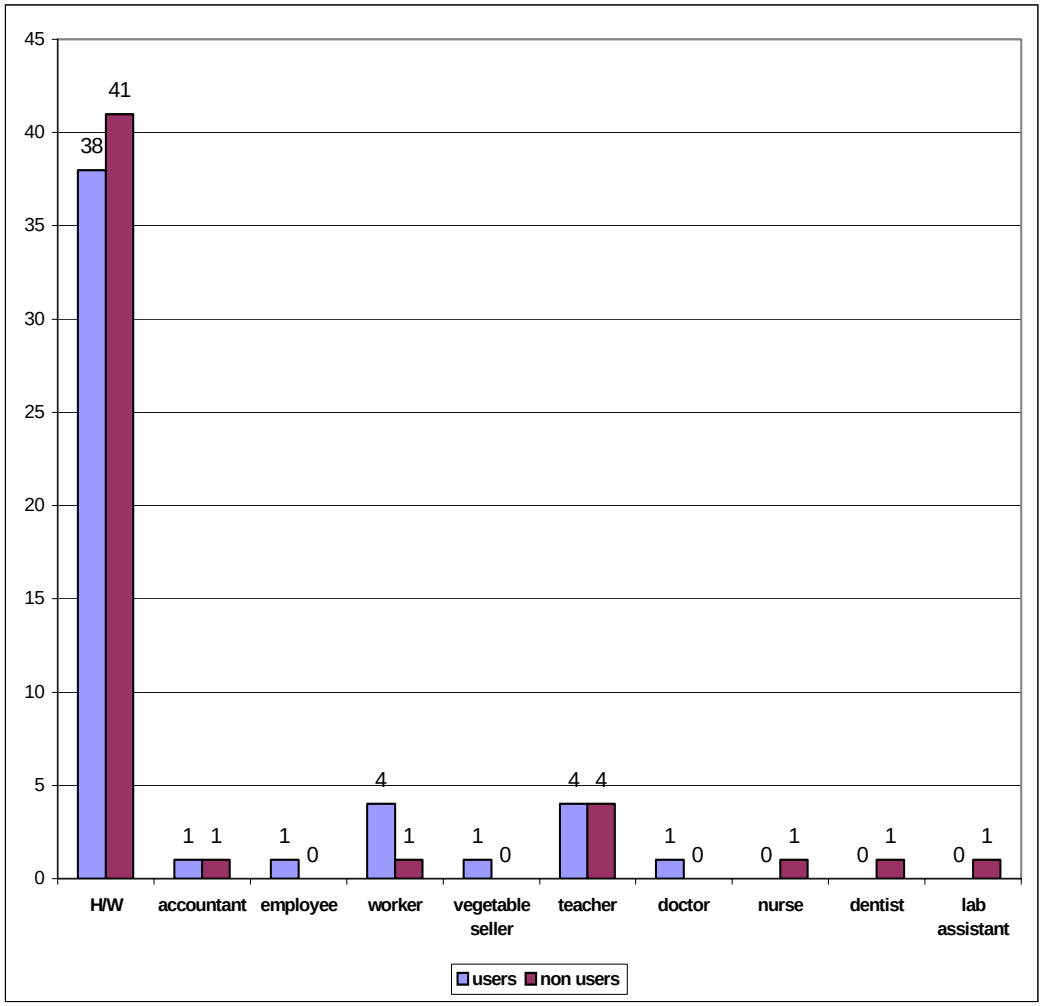

Figure 2: Distribution of study population by occupation.

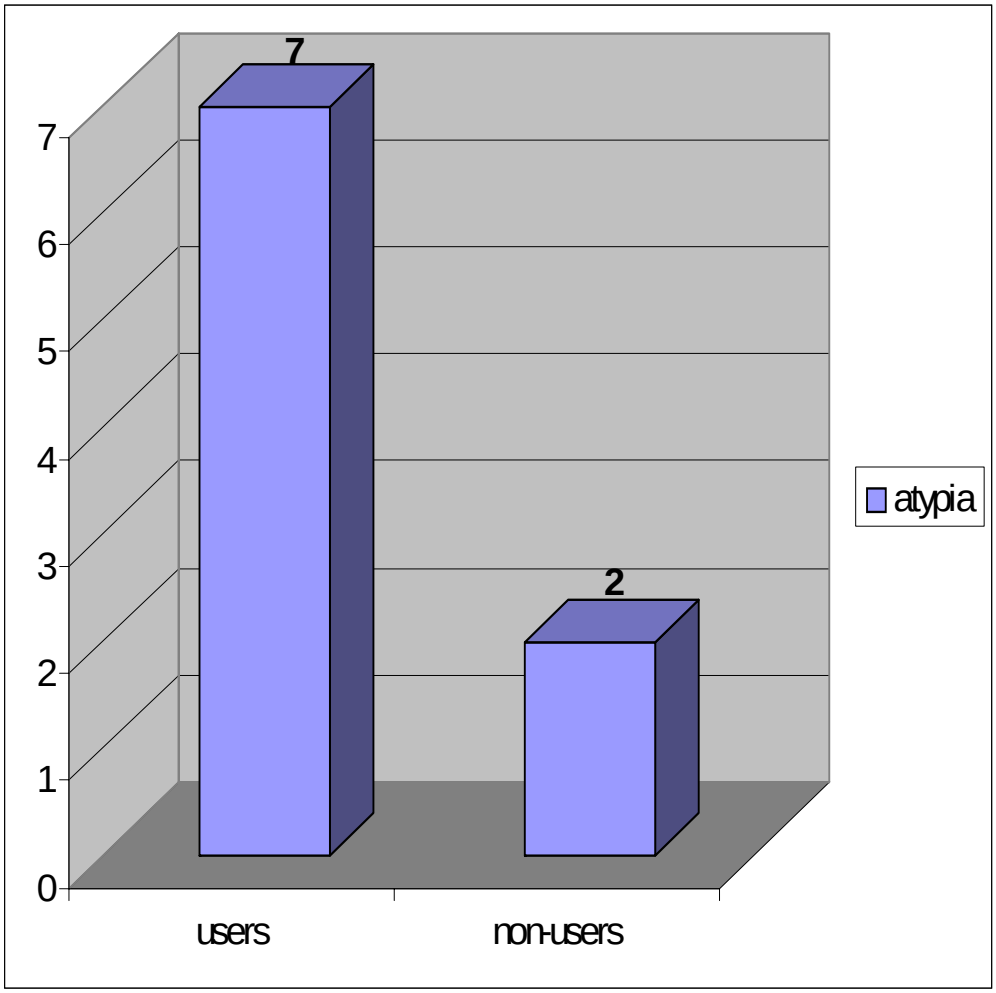

Figure 3: Description of cytological atypia by exposure.

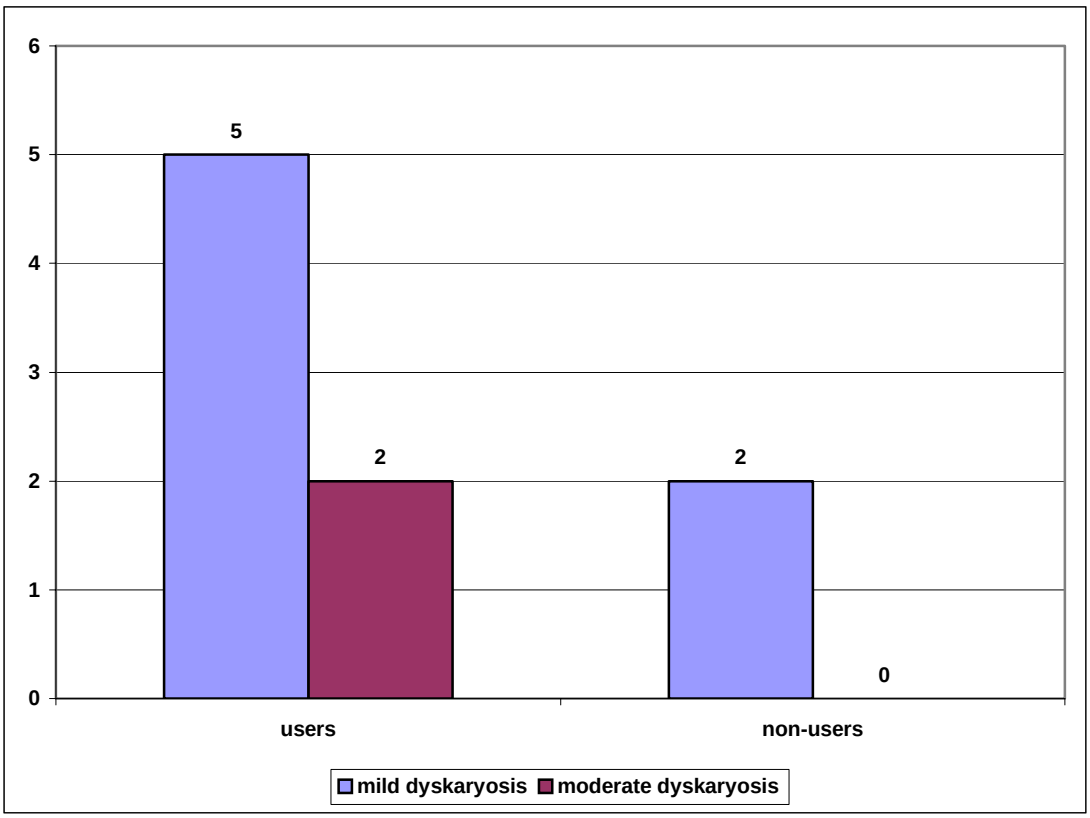

Figure 4: Description of the degree of cytological atypia by exposure.

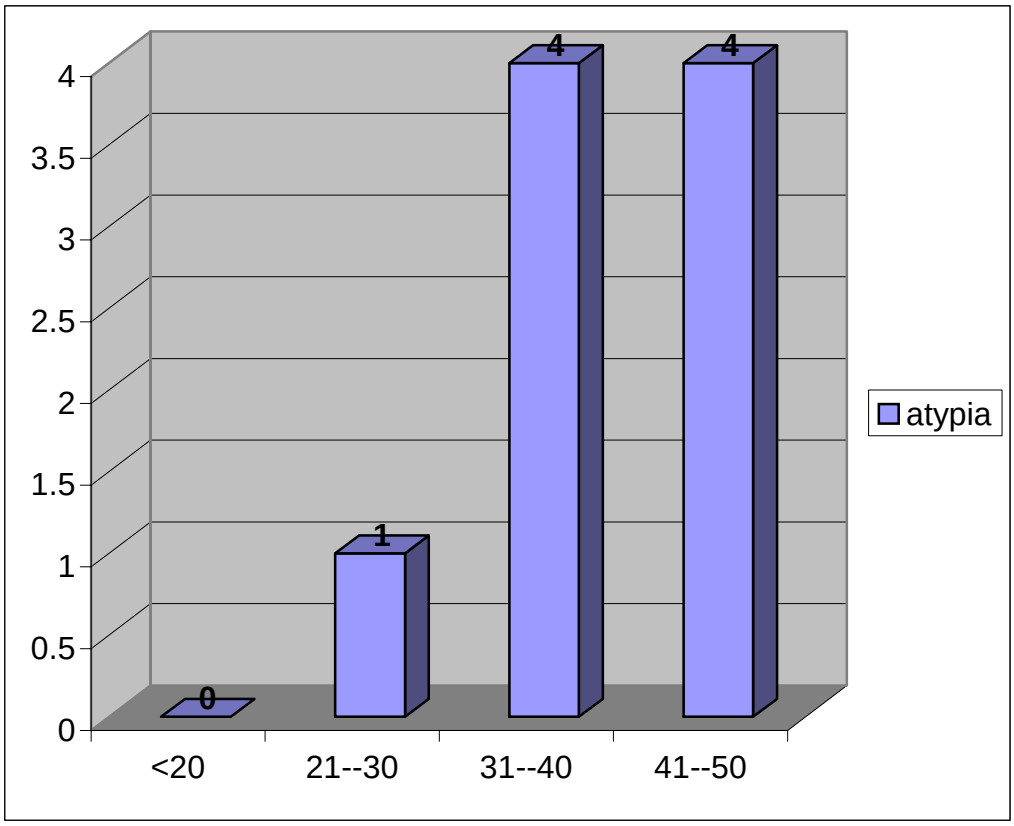

Figure 5: Description of atypia by age (in years).

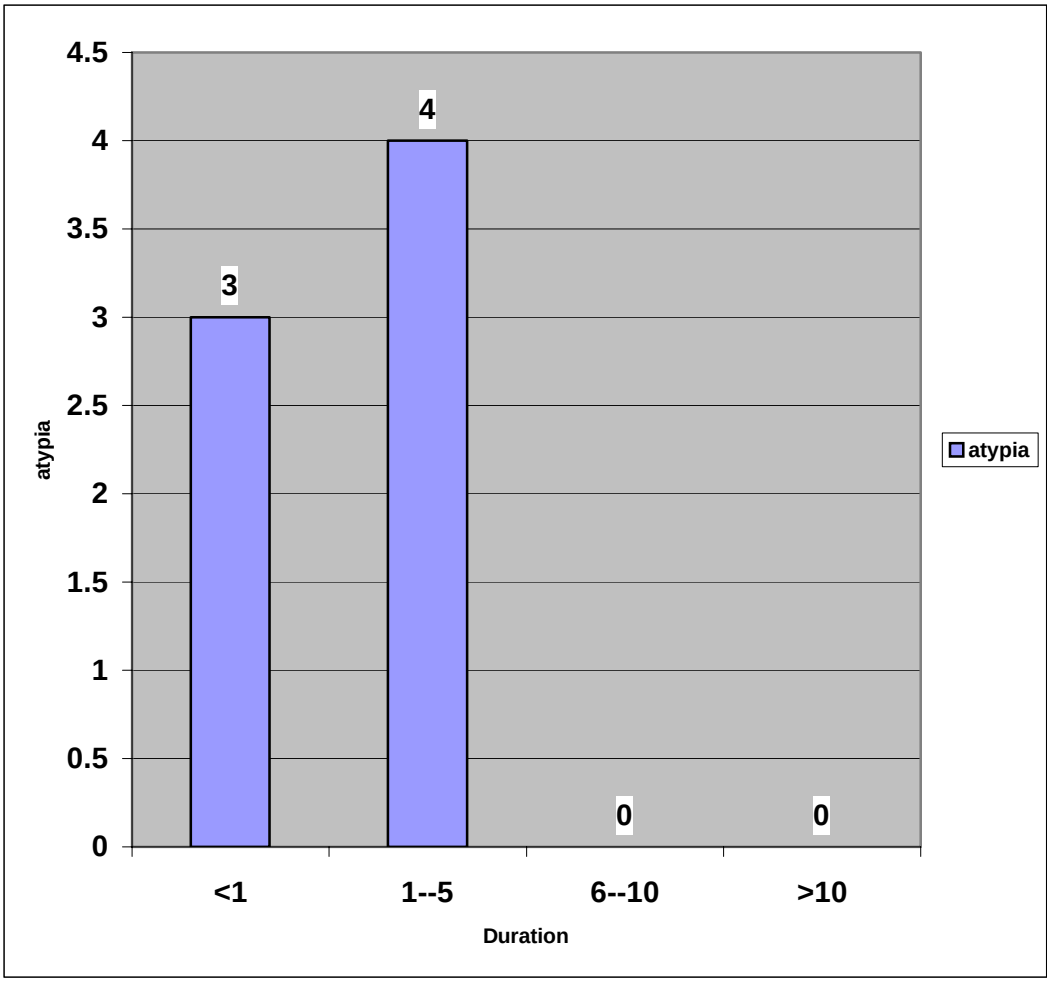

Figure 6: Description of cytological atypia by duration of OC use.

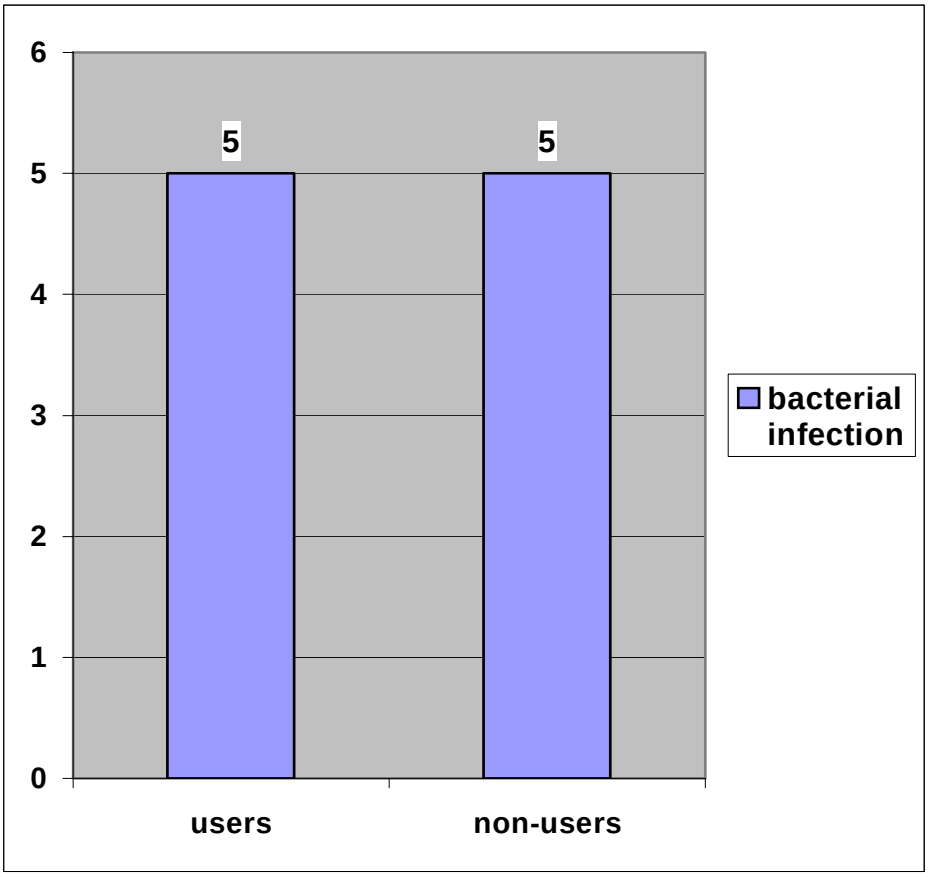

Figure 7: Description of bacterial infection by exposure

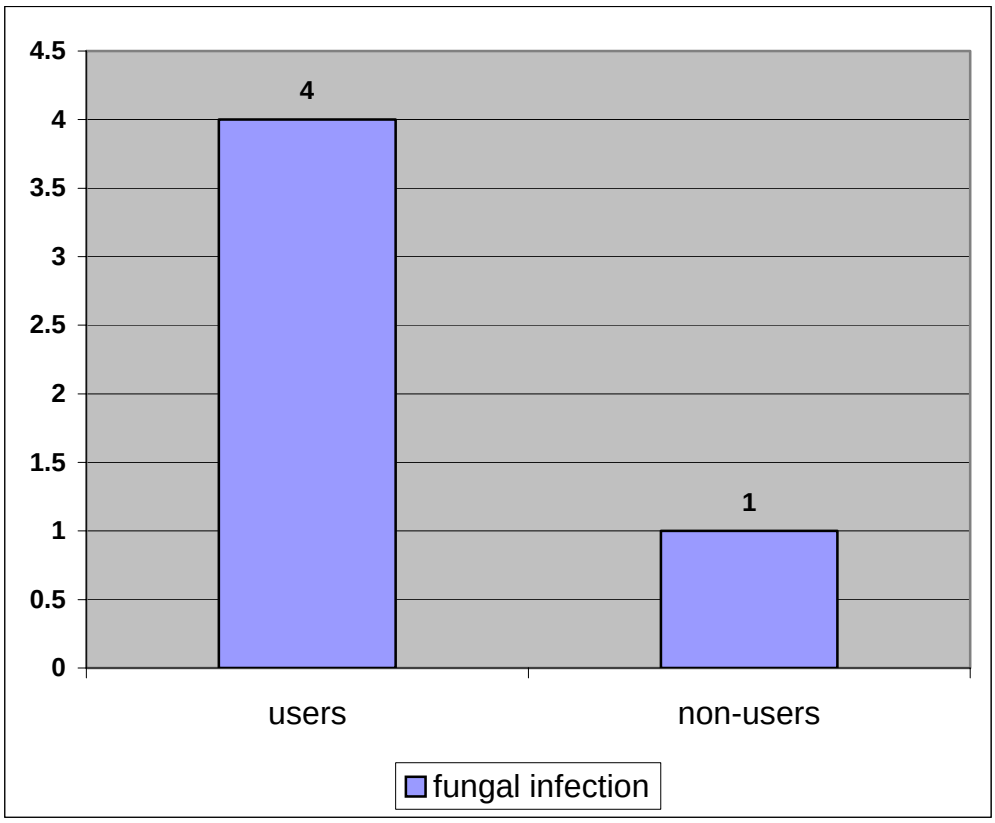

Figure 8: Description of fungal infection by exposure.

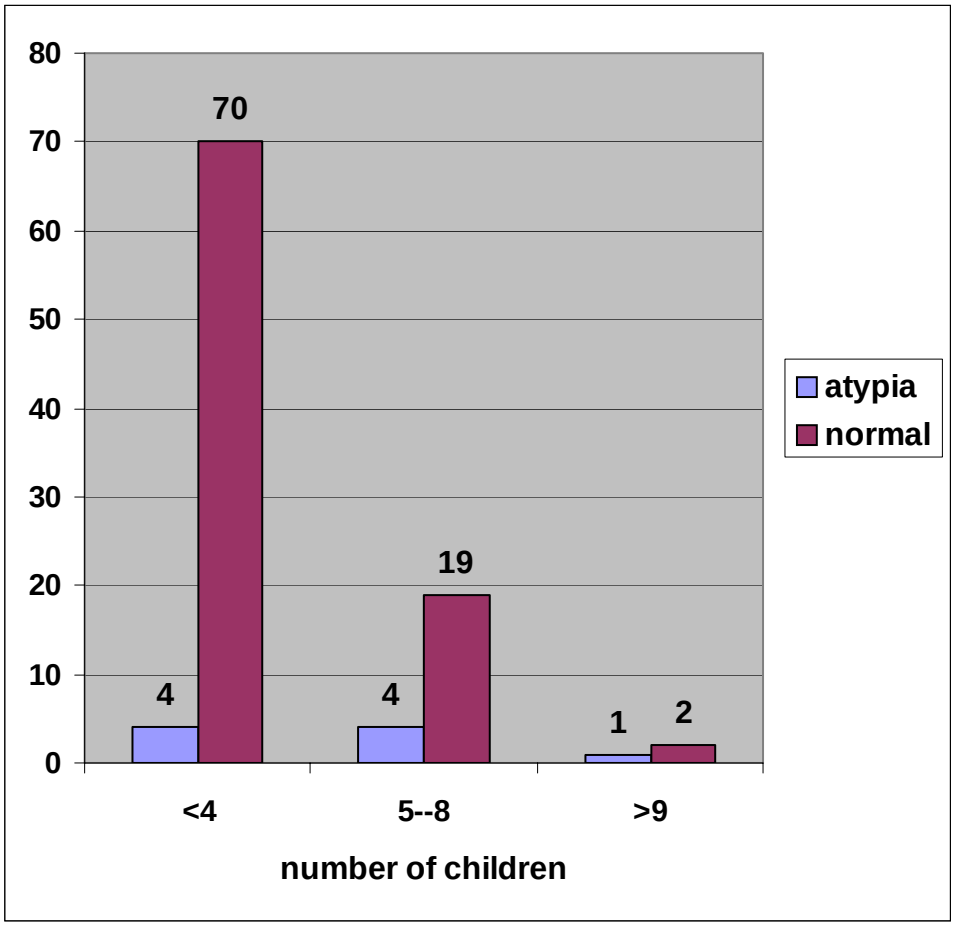

Figure 9: Description of cytological atypia by frequency of childbirth

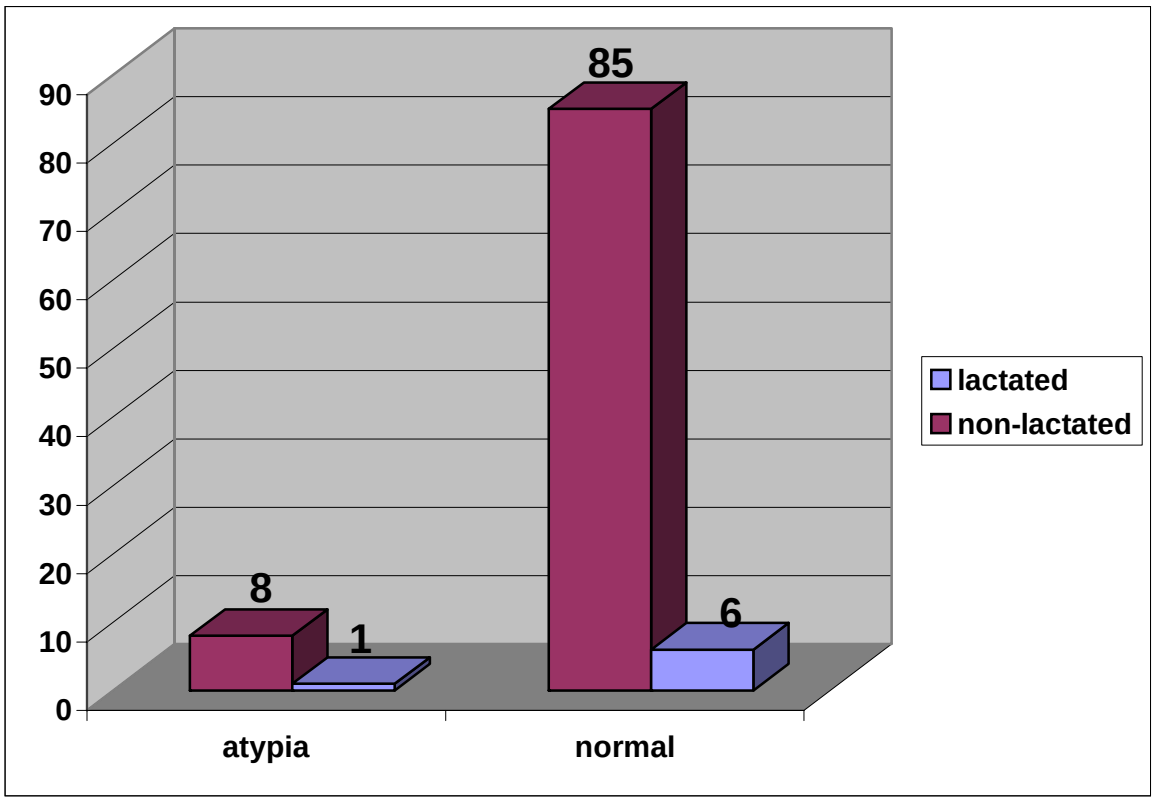

Figure 10: Description of cytological atypia by lactation.

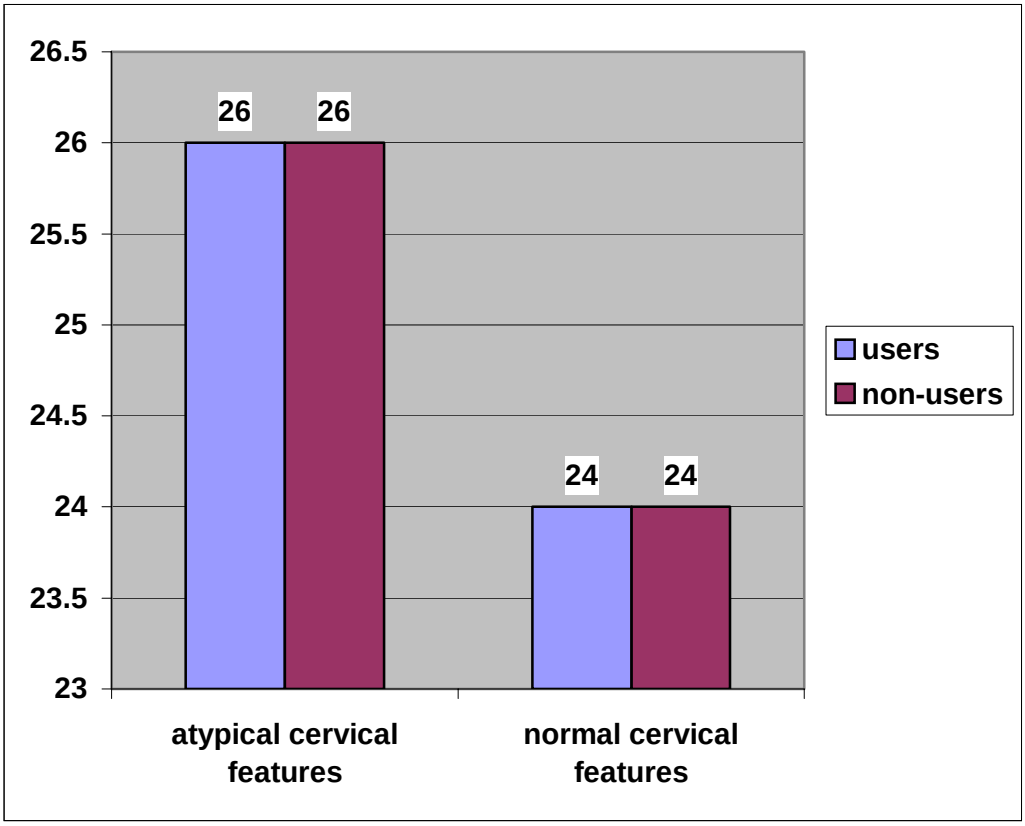

Figure 11: Description of cervical features by exposure.

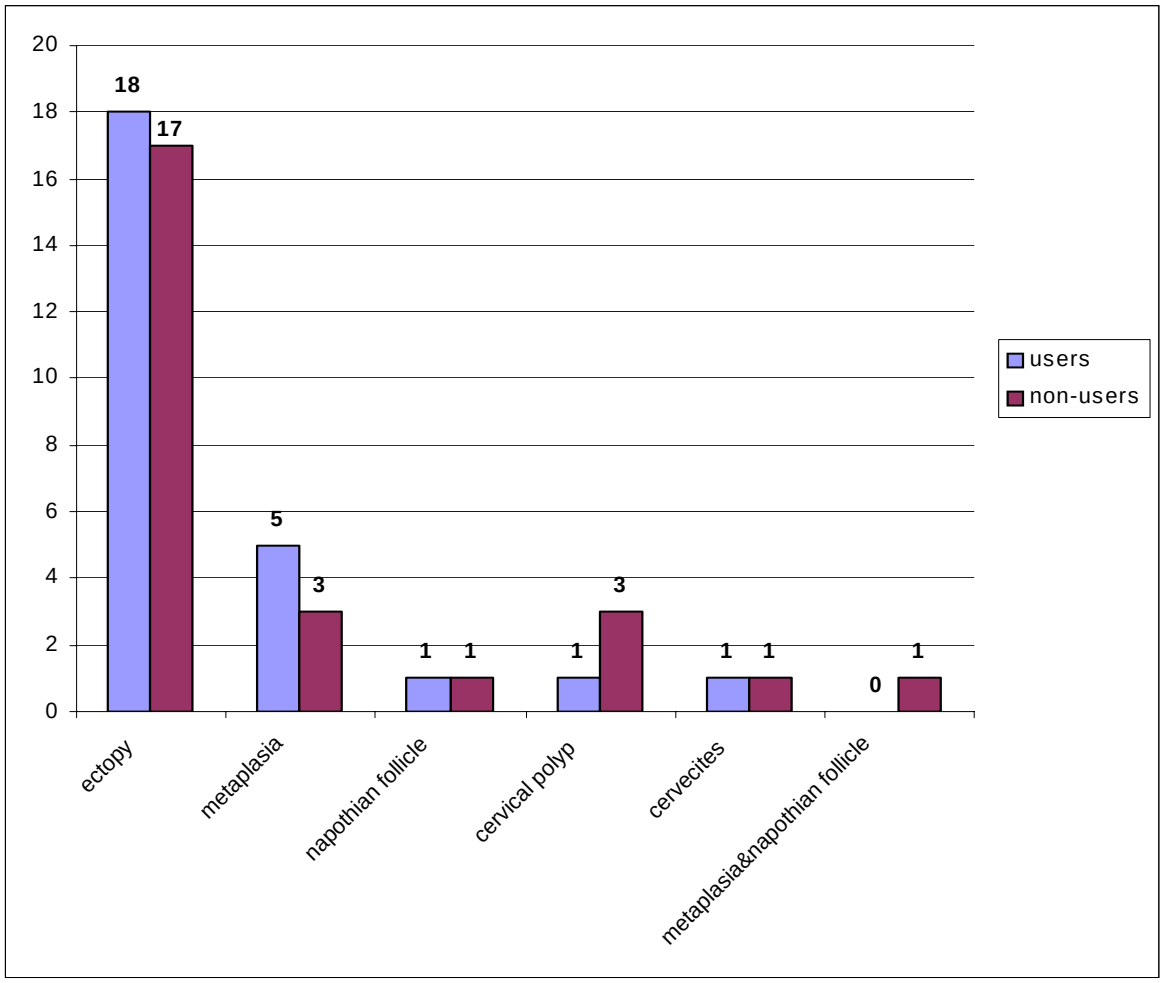

Figure 12: Description of atypical cervical features by exposure.

Photo.13. Cervical smear. Pap. x40. Mature superficial squamous cells. Note the tiny pyknotic nuclei and thin flat transparent cytoplasm, which stained a delicate pink.

Photo.14. Cervical smear. Pap. x40. Intermediate cells and superficial cell. Note the larger, vesicular nucleus of intermediate cell

Photo.15. Cervical smear. Pap. x40. Parabasal cells of various size. Note that the nuclei are vesicular.

Photo.16. Cervical smear. Pap. x40. Endocervical cells. Note the columnar shape, the finely vaculated cytoplasm and round nuclei.

Photo.17. Cervical smear. Pap. x40. Endocervical cells. Note the honeycomb appearance.

Photo.18. Cervical smear. Pap. x40. Metaplastic cells.

Photo.19. Cervical smear. Pap. x40. Histiocytes&lymphocytes. Note the vaculated cytoplasm and kidney shape nuclei of histiocytes.

Photo. 20. Cervical smear. Pap. x40. Polymorphnuclear leukocytes.

Photo. 21. Cervical smear. Pap. x40. Lactobacilli (Doderlein). Note the characteristic slender rods.

Photo. 22. Cervical smear. Pap. x40. Nuclear karyorrhexis. Fragments of nuclear material are seen in the cytoplasm of some cells (karyorrhexis).

Photo. 23. Cervical smear. Pap. x40. “Clue cells”: a superficial squamous cell covered by innumerable rods of Gardnerella vaginalis, giving it a peculiar grayish appearance.

Photo. 24. Cervical smear. Pap. x40. Monilia (Candida) albicans. Note budding.

Photo. 25. Cervical smear. Pap. x40. Monilia (Candida) albicans. Note pseudohyphae.

Photo. 26. Cervical smear. Pap. x40. Intermediate squamous cells with minor nuclear abnormalities (mild dyskaryosis). Note the slight enlargement and hyperchromasia of nuclei.

Photo. 27. Cervical smear. Pap. x40. Mild dyskaryosis of intermediate cell. Note nuclear enlargement and hyperchramasia.

Photo. 28. Cervical smear. Pap. x40. Mild dyskaryosis of intermediate squamous cells. Note the slight increasing of nuclear sizes and unequal chromatin distribution.

Photo. 29. Cervical smear. Pap. x40. Moderate dyskaryosis of the parabasal cells. The nuclei contains most of cancer features and displays marked hyperchromasia. Note the binuleation.

Photo. 30. Cervical smear. Pap. x40. moderate dyskaryosis of the parabasal cell. Note nuclear enlargement, hyperchromasia and irregularity of outline.

Photo.31. Cervical smear. Pap. x40. Moderate dyskaryosis of parabasal squamous cells. Note the large irregular hyperchromatic nucleus.

CHAPTER SIX

DISSCUSSIONS

Cervical cancer is the second female cancer both worldwide and in the Sudan (NIH, 1996, WHO 2003). Many studies link the use of contraceptive pills to cervical cancer (Smith, 2003).

This study aimed of assessing the role of OC pills as a risk factor for occurrence of cervical cytological atypia.

In the present study, of the 100 study subjects cytological atypia was detected among 7(14%) of the cases and only 2(4%) of the controls. These

findings showed that OC pills use is a risk factor for occurrence of cervical cancer, and this was found to be statistically significant (P< 0.006).

These findings support many previously published studies. Ljuca, et al. (2000) found a high significant positive relationship between OC use and precancerous and early cancerous lesions of the cervix. The study by Liu, et al. (1967) showed that abnormal cytologic finding in gynecologic smears were more common among patients using OCs than among control patients.

Morrison, et al. (2003), noted a significant increase in the diagnosis of low-grade dyskaryosis among OC users. They investigated Thin Prepared cytological smears from 1000 women on OC and compared them to 1200 smears from women not taking birth control pills. The reported rate of dyskaryosis among users was more than twice that of the women not taking birth control pills (9 percent vs 4 percent) (P=0.02).

Favre, et al. (1979) investigated cytological smears from 1685 patients on OC pills and compared them to 1000 smears from women not on OC. They were between 19-45 years and had been treated for periods going from 2 months to 10 years. Dyskaryosis was found in 3.6% of OC patients, and in 1.4% of controls.

However, the results reported in a study by Berget, et al. (1974) oppose our current findings. They found that when comparing OC users to those non-users, no statistically significant difference was observed. Wherein 13,125 women were studied; of whom 1471 had used OC pills for an

average of 24 months. Abnormal cytological findings were disclosed in 446 women.

This difference may be due to a small number of OC pills users (1476) compared to non-users (11654). In our study, equal numbers of users and non-users were studied. Therefore, our study may be more indicative.

According to duration of OC pills use, duration of 1-5 years showed the highest frequency of cervical atypia 4(57%), followed by <1 year 3(43%). However, most of the cases were aggregating at these 2 durations ranges, 25 and 21 for duration of 1-5 and <1year respectively. Taking into account these findings, the effects of OCs on cervical cells seemed to be earlier following the OC pills use. The exact relationship between long duration of use of more than 5 years and cytological atypia was not clear in this study due to small number of cases in duration ranges of more than 5 years.

These findings support the findings by Chen (1996), who found that the risk of cervical cancer increased after only 1- 6 months of OC pills use. An other study by Schiffman (2003), found that the relative risk of cervical cancer increased with increasing duration of use of OC pills. The relative increases in risk were 10% for less than or equal 5 year use.

These findings also support the results obtained by Negrini (1990), he found that the risk of dyskaryosis increased with duration equal or greater than 5 years.

Furthermore, most of cytological atypias were detected among elder age individuals. There were 7 atypical changes among age ranges more than

35 years compared to only 2 among age less than 35 years. These findings indicating that the risk of cervical cancer is increasing with age, and this was found to be statistically significant (P<0.007).

These findings support other findings obtained by Ahmad, et al. (2002), who found that out of 526 women, 23 cases of dyskaryosis were detected. Seventeen cases were among age groups >31 years, compared to 6 cases among age groups less than 31 years.

There was no significant relationship between the use of OC pills and the rate of bacterial genital tract infections. These findings support the findings by Hawkins, et al. (1999); they compared the rates of genital tract infections and the OC pills among a random sample of 800 women. Bacterial genital tract infections did not differ significantly in use of OC pills.

An other study by Fabiani (1983), analyzed 1432 smears from OC and IUD users and non-users. The rate of inflammatory smears was found to be the same for all 3 populations studied.

Nevertheless, fungal infections were detected among 4 of OC pills users compared to only one fungal infection among non-users. These findings in our series indicating that the use of OC pills increases the rate of fungal infection and this was found to be statistically significant (P<0.001). The increased Candida albicans incidence in OC users may be due to the effect of the estrogen, which is the hormone responsible for increased binding of insulin to serum protein. This will increase glucose concentrations, which influence the vaginal epithelium and the acidity of the vagina. This acidity

increases the rate of fungal infection. These findings support the findings obtained by Walsh, et al. (1968), they noted a significant increase in Candida albicans infections among OC users. They compared 42 users women to 14 non-users. Candida albicans was found among 24 of users compared to none among non-users. Sharief (1998) found that the Candida spp infections were higher among women using OC (25%) compared to (14%) among non-users. And this showed statistically significant differences ($P<0.05$).

Yeast cultures were made by Jenson, et al. (1970) from vaginal swabs of 80 users and 158 non-users. In OC users group, 15% had Candida albicans compared to 5% in non-users group. The difference was significant at 5% level.

In this study, the rate of cervical cytological atypia was apparently elevated among women who have more frequencies of child bearing. This increase may be related to the mechanics and stress of childbirth such as stretching and tearing of the cervix.

These findings support the study by Ahmad (2002), among 25 cases of dyskaryosis, 15(60%) were among women with more than 4 children, compared to 10(40%) among those with less than 4 children.

Another study by Munoz (2002), compared nilliparous women with those with 3 or 4 full-term pregnancies had 2.55 times the risk of developing cervical cancer, and those with 7 or more births had 3.82 times the risk.

The relationship between lactation and cervical cytological atypia was not so clear, as 93% of our study subjects were non-lactated, compared to only 7% lactated women.

The normal and abnormal gross cervical features were similar among cases and controls. Among users, cervical ectopy was the most frequently observed change in the cervix. However, the rate of this feature did not differ significantly between the two groups. This supported the finding obtained by Prilepskaia (1991), he found that cervical ectopia can occurs under use of OC pills for 6-12 months but it vanishes after discontinuation.

CHAPTER SEVEN

CONCLUSION & RECOMMENDATIONS

Oral contraceptive pills use is an important factor that induces cytological atypia in the cervix mainly dyskaryosis, which is pre-malignant lesion. These changes appear after a short period of OC pills use and increase with the increase of age.

OC pills users should undergo continuous screening program using exfoliated cytology.

CHAPTER EIGHT

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APPENDIX I

University of Khartoum
Faculty of medical laboratory sciences
Histopathology & Cytology department

QUESTIONNAIRE

Number……. Date……...
Name……. Age…….
Occupation…… Marital status……
Residence…….. Number of children…

OC user ☐ Non-OC user ☐

Type of OC pills:

Estr. ☐ Estr. -Proges. ☐

Duration of OC pills use:

<1 year ☐ 1-5 years ☐ 6-10 years ☐ >10 ☐

Infection ☐ Lactation ☐ Pregnancy ☐

Gross features of the cervix:
……
……
……
……

Microscopical findings:
……
……

……
……

APPENDIX II

Materials:

- Ayres spatula.
- Glass slides.
- Plastic slide containers.
- Disposable gloves.
- Cover glass.
- Coplin jars.
- Microscope.
- Filter paper.
- Absolute alcohol.
- Distilled water (D.W).
- Xylene.
- D.P.X.

1- Staining solution (Papanicolaou stain):

Harris`s haematoxylin:

Haematoxylin……..... 5 g.

Ethanol……...50 ml.

Potassium alum…….100 g.

D.W……1000 ml.

Mercuric oxide…….2-5 g.

Glacial acetic acid…..40 ml.

Orange G 6:

Orange G (10% aqueous)…….50 ml.

Alcohol……..…950 ml.

Phosphotungstic acid……..……..0-15 g.

EA 50:

0.04 M light green SF……10 ml.

0.3 M eosin Y……20 ml.

Phosphotungstic acid……2 g.

Alcohol……750 ml.

Methanol……..……..…250 ml.

Glacial acetic acid……20 ml.

Filter all stains before use.

2- 95% ethyl alcohol:

Absolute ethanol……..……..95 ml.

D.W……..….5 ml.

3- 0.5% aqueous HCL:

HCL……..……0.5 ml.

D.W……..……99.5 ml.

4- Alkaline tap water:

Ammonia……..…….0.1 ml.

D.W……99.9 ml.