Alama KA and Abkar AD, J Cytol Tissue Biol 2019, 6: 021
DOI: 10.24966/CTB-9107/100021

HSOA Journal of Cytology and Tissue Biology

Prospective Research

# Detection of Aberration and Genetic Changes in Post-menopausal Women Presented with Bleeding Using Pap Smear, Immunocytochemical and Molecular Studies

**Khalid Ahmed Alama[1]* and Adam Daood Abkar[2]**

[1]*Senior Clinical Cytologist at Dubai Hospital, Dubai, UAE*

[2]*University of Gezira, Gezira, Sudan*

## Abstract

**Objective:** Two of the most common malignancies of the female genital tract are endometrial and cervical cancers, these are highly prevalent cancers, and their further study has the potential for significant beneficial impact on women's health on an international level. The p53 tumor suppressor gene has received considerable attention in endometrial cancer. Mutations in p53 are found in approximately 10%-20% of all endometrial carcinomas, the majority occurring in high-grade tumors. As many as 50% of all human tumors contain p53 mutants. In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: growth arrest, DNA repair and apoptosis (cell death). p53 also known as TP53 or tumor protein is a gene codes a protein that regulates the cell cycle and apoptosis, hence functions as tumor suppression. p53 has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation, it is located on the seventeenth chromosome (17p13.1) and molecular weight 53 Kilodalton fraction of cell protein. Abnormalities of TP53 have been well described in various malignancies and germ-line mutations are associated with Le Fraumeni syndrome. The mutant p53 protein is non-functional but resists degradation and accumulates thereby acting as a dominant negative inhibitor of the wild-type p53. The accumulated mutant protein can be demonstrated immunohistochemically. The p53 signature, which (although morphologically unremarkable) displays diffuse and strong p53 nuclear staining, has been proposed to be a precursor of serous endometrial and/or endndocervicalintraepithelial neoplasia. We examined the overexpression of p53 in postmenopausal women with vaginal bleeding in exfoliated endometrial and endocervical cells collected by endo and ectocervical brushing.

**Methods:** Liquid Base (LBC) PAP smears of 70 women with postmenopausal bleeding with normal Cytomorphological findings ranging between Negative for Intraepithelial Lesion (NILM) and Reactive Cellular Changes (RCC), including 5 samples collected from postmenopausals without bleeding used as a control, were evaluated in this study. Menstruating women were excluded from this study. Expressions of p53 were immunohistochemically examined and the findings confirmed using molecular analysis (PCR). Overexpression of p53 was categorized as weak to strong in more than 18% of the samples.

**Results:** Strong overexpression of p53 was observed in 15 samples (10.5%) and 12 samples (8.4%) showed weak p53 overexpressionin Immunohistochemical studies. Molecular analysis (PCR) used to confirm the results and it showed 13 Positive using gel electrophoresis and read under Ultra Violet (UV) light. Twelve out of Fifteen samples which were strongly positive in immunomarker stain and One out of Twelve weak positive samples in immunomarker stain showed positive mutational changes in molecular study. Postmenopausals with bleeding exhibited significantly overexpression of P53 (P = 0.001).

**Conclusion:** Overexpression of p53 may be responsible for the high proliferative activity of postmenopausal endometrial intraepithelial neoplasia and/or endocervical intra epithelial neoplasia.

***Corresponding author:** Khalid Ahmed Alama, Senior Clinical Cytologist at Dubai Hospital, Dubai, UAE, Tel: +971 504182550; E-mail: khalidalama@gmail.com

**Citation:** Alama KA, Abkar AD (2019) Detection of Aberration and Genetic Changes in Post-menopausal Women Presented with Bleeding Using Pap Smear, Immunocytochemical and Molecular Studies. J Cytol Tissue Biol 6: 021.

**Received:** May 14, 2019; **Accepted:** June 03, 2019; **Published:** June 10, 2019



## Introduction

Two of the most common malignancies of the female genital tract are endometrial and cervical cancer. They are separate entities with different epidemiology, pathogenesis, and histology, but are linked by their origin in the uterus. Although they have garnered less attention in developed countries than ovarian carcinoma, it is important to recognize that endometrial carcinoma is the most common female genital tract malignancy in the United States, and that cervical carcinoma is the second most common cancer in women worldwide. Thus, these are highly prevalent cancers, and their further study has the potential for significant beneficial impact on women's health on an international level [1]. Endometrial cancer is the most common malignancy in females' gynecological cancer mostly affecting women in the post-menopausal age group [2]. It arises from the endometrium, it is the result of the abnormal growth of cells that have the ability to invade or spread to other parts of the body [3]. The first sign is most often vaginal bleeding not associated with a menstrual period. Other symptoms include pain with urination, pain during sexual intercourse, or pelvic pain [3]. Some risk factors are related to reproduction, such as early age at menarche, late age at menopause and nulliparity, while others are oestrogen-related as in conditions such as the polycystic ovarian syndrome. Use of unopposed oestrogen replacement therapy

is associated with an increased risk. Obesity, diabetes and hypertension and smoking increase the risk of endometrial cancer. While, low-fat diets and physical exercise and use of the combined oral contraceptive pill is associated with a decreased risk. All of these possibly exert their effects by various indirect influences on oestrogen levels, thus influencing the level of stimulation of the target endometrial epithelium. Inherited forms of endometrial cancer exist [4,5]. Worldwide, cervical cancer is both the fourth-most common cause of cancer and the fourth-most common cause of death from cancer in women. In 2012, an estimated 528,000 cases of cervical cancer occurred, with 266,000 deaths. This is about 8% of the total cases and total deaths from cancer. About 70% of cervical cancers occur in developing countries. In low-income countries, it is the most common cause of cancer death [6].

Post-menopausal bleeding accounts for 9-10% of gynecological complaints. 10% of patients presenting with post-menopausal bleeding are diagnosed to have carcinoma of endometrium. At the same time 90% of the patients with carcinoma of endometrium present with postmenopausal bleeding, therefore it is important to evaluate all patients with post-menopausal bleeding to rule out malignancies of the genital tract. Other causes of post-menopausal bleeding including atrophic endometrium (60%), endometrial hyperplasia (10%), carcinoma cervix (10%) and other rare conditions (10%) [7,8].

## Mutations in mismatch repair genes

Mutations in mismatch repair genes result in hereditary non-polyposis colorectal cancer, which confers a lifetime risk of bowel cancer between 60-80% and an endometrial cancer risk of up to 60%. Genetic disorders can also cause endometrial cancer. Overall, hereditary causes contribute 2-10% of endometrial cancer cases [6]. Lynch syndrome, an autosomal dominant genetic disorder that mainly causes colorectal cancer, also causes endometrial cancer, especially before menopause. Women with Lynch syndrome have a 40-60% risk of developing endometrial cancer, higher than their risk of developing colorectal (bowel) or ovarian cancer [9,10]. Ovarian and endometrial cancer develops simultaneously in 20% of people. Women with a family history of endometrial cancer are at higher risk [3]. There is an apparent link between the use of tamoxifen and endometrial cancer [9]. The inherited genetic condition Cowden syndrome can also cause endometrial cancer. Women with this disorder have a 5-10% life time risk of developing endometrial cancer, compared to the 2-3% risk for unaffected women [11]. Common genetic variation has also been found to affect endometrial cancer risk in large-scale genome-wide association studies. Sixteen genomic regions have been associated with endometrial cancer and the common variants explain up to 7% of the familial relative risk [12]. The molecular pathogenesis of endometrial carcinoma remains poorly understood. However, as in other malignancies, such as colorectal cancer, the transition from normal endometrium to carcinoma is thought to involve a stepwise accumulation of alterations in genes, favouring cell proliferation and inhibition of apoptosis and angiogenesis [13]. Many studies prove a strong relation between Proliferating Nuclear Antigen (PCNA) and p53 protein accumulation [14].

As many as 50% of all human tumors contain p53 mutants. In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: growth arrest, DNA repair and apoptosis (cell death) [15]. P53 also known as TP53 or tumor protein is a gene codes a protein that regulates the cell cycle and apoptosis, hence functions as tumor suppression. p53 has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation, it is located on the seventeenth chromosome (17p13.1) and molecular weight 53 Kilodalton fraction of cell protein [16]. Abnormalities of TP53 have been well described in various malignancies and germ- line mutations are associated with Le Fraumeni syndrome. The mutant p53 protein is non-functional but resists degradation and accumulates thereby acting as a dominant negative inhibitor of the wild-type p53. The accumulated mutant protein can be demonstrated immunohistochemically. Wild type p53 protein has a short half-life; it is generally undetectable in normal cells, whereas mutant p53 protein is more stable and often reaches immunohistochemically detectable levels [17]. Mutations of the p53 gene are a frequent and characteristic finding in Type II serous tumour with positive immunohistochemistry reported in 71%-85% of tumour and are considered to be an early event in tumorigenesis [18]. This is supported by concordant p53 staining in multiple biopsies from different areas of individual tumour and by the finding of positive immunohistochemical results in Endometrial Intraepithelial Carcinoma (EIC), the putative precursor of serous carcinoma [19].

The aim of the study was to find out any possibility of gene mutational changes in postmenopausals with bleeding symptoms as an alert and early prediction for an occult endometrial or endocervical mutational genetic changes.

## Problem identification and justification

Endometrial cancer is known to be more common in postmenopausal women and the incidence rates were 4 to 20 times higher in women age 50 and older than in women under 50. Early detection improves the chances that cancer can be treated successfully. But some endometrial cancers may reach an advanced stage before signs and symptoms can be noticed [20]. Given the rise in endometrial cancer incidence and mortality, raises the importance of managing postmenopausal bleeding to optimize the benefit of early detection approaches while avoiding unnecessary harms.

## The role of P53 mutation in cancer development

### DNA damage and repair

TP53 gene encodes Proteins (P53) that binds to DNA and regulates gene expression to prevent mutations of the genome [21]. Genetic evidence and experimental data strongly supports that mutation of p53 gene is probably one of the most important factors to initiate the endometrial carcinogenesis. The development of cancer is a multistep process with accumulation of somatic gene mutation, including activation of oncogenes and/or inactivation of tumor suppressor genes. Support that mutation of p53 tumor suppressor gene plays an important role in endometrial carcinogenesis [22,17]. Mutations of p53 gene in endometrial cancers are diverse. Each p53 mutation seems to be unique. Comparing specific p53 gene mutation in different sites of tumors has been successfully applied to differentiate clonal (metastatic) process from synchronous or independent growth [23].

P53 plays a role in regulation or progression through the cell cycle, apoptosis, and genomic stability by means of several mechanisms:

- It can activate DNA repair proteins when DNA has sustained damage. Thus, it may be an important factor in aging [24]

- It can arrest growth by holding the cell cycle at the G1/S regulation point on DNA damage recognition if it holds the cell here for long enough, the DNA repair proteins will have time to fix the damage and the cell will be allowed to continue the cell cycle
- It can initiate apoptosis (i.e., programmed cell death) if DNA damage proves to be irreparable
- It is essential for the senescence response to short telomeres

**Incidence and mortality**

Evaluation of women with postmenopausal bleeding has the potential to capture as many as 90% of endometrial cancers, according to the results of a meta-analysis published recently in *JAMA Internal Medicine.* Ninety-one percent of women with endometrial cancer had postmenopausal bleeding. There was no significant difference in the prevalence of postmenopausal bleeding by cancer stage. Looking at different geographic regions, the researchers found that the prevalence of postmenopausal bleeding was 94% in North America and 90% in Western Asia and Eastern Asia. Ninety-one percent of women with endometrial cancer had postmenopausal bleeding [25]. Estimated new cases and deaths from cancer of the uterine corpus includes the endometrium In the Unites states in 2019 among 61.880 new cases there were 12.160 deaths: [20]. Cervical cancer incidence is highly concentrated in the southern part of the hemisphere. The highest concentration is in central South America which constitutes about 71,000 cases a year, and sub-Saharan Africa constitutes 78,000 a year, followed by India and Southeast Asia, which also has 260,000 cases occurring in a year. The lowest incidence for this cancer occurs in North America, Europe, and Australia this is really a result of programs in those countries which aim to attract women for cervical cancer screenings through Pap smears and subsequently identify precancerous cells which are treated to protect the women from getting cervical cancer.

## Objectives

### General objectives

The objective of this study was to clarify the possibility of genes mutation in postmenopausals with bleeding who has a negative Pap smear result, as an early prediction for endometrial and endocervical cancer.

### Specific objectives

- To determine the role of cytological examination of Papanicolaou (Pap) smears in diagnosing endometrial and endocervical cancer and precancer changes
- To determine the role of p53 overexpression in negative Pap smears of postmenopausals with bleeding as an early prediction of endometrial and endocervical cancer.

## Literature Review

Endometrial cancer appears most frequently between the ages of 50 and 65 [26]. Overall, 75% of endometrial cancer occurs after menopause. Women younger than 40 make up 5% of endometrial cancer cases and 10-15% of cases occur in women under 50 years of age. This age group is at risk for developing ovarian cancer at the same time. [26]. The worldwide median age of diagnosis is 63 years of age; [27]. In the United States, the average age of diagnosis is 60 years of age. White American women are at higher risk for endometrial cancer than black American women, with a 2.88% and 1.69% lifetime risk respectively [28]. Japanese-American women and American Latina women have a lower rate and Native Hawaiian women have higher rates [29]. As of 2014, approximately 320,000 women and over 380,000 new cases in 2018 are diagnosed with endometrial cancer worldwide each year, and 76,000 die, making it the sixth most common cancer in women [6]. It is more common in developed countries, where the lifetime risk of endometrial cancer in people born with uteri is 1.6%, compared to 0.6% in developing countries [30]. It occurs in 12.9 out of 100,000 women annually in developed countries [31].

In the United States, endometrial cancer is the most frequently diagnosed gynecologic cancer and in women the fourth most common cancer overall [9] representing 6% of all cancer cases in women. In that country, as of 2014 it was estimated that 52,630 women were diagnosed yearly and 8,590 would die from the disease [28].

Northern Europe, Eastern Europe, and North America have the highest rates of endometrial cancer, whereas Africa and West Asia have the lowest rates. In Asia 41% of the world's endometrial cancer diagnoses in 2012, whereas Northern Europe, Eastern Europe, and North America together comprised 48% of diagnoses [6]. Unlike most cancers, the number of new cases has risen in recent years, including an increase of over 40% in the United Kingdom between 1993 and 2013 [30]. Some of this rise may be due to the increase in obesity rates in developed countries [31]. Increasing life expectancies, and lower birth rates [9]. In the UK, approximately 7,400 cases are diagnosed annually, and in the EU, approximately 88,000 [27]. There were over 500,000 new cervical cancer cases in 2018, representing 6.6% of all female cancers. Approximately 90% of deaths from cervical cancer occurred in low- and middle-income countries. The high mortality rate from cervical cancer globally could be reduced through a comprehensive approach that includes prevention, early diagnosis, and effective screening and treatment programmers. There are currently vaccines that protect against common cancer-causing types of human papilloma virus and can significantly reduce the risk of cervical cancer. 90% of CIN lesions and cervical cancer are associated with HPV.

## Materials and Methods

### Study area

The study is a prospective cross sectional study of endocervical samples collected from postmenopausal patients with bleeding in Gynecological clinics and submitted to the medical laboratory.

### Study design

Cross sectional prospective study

### Study area and period

The study conducted in United Arab Emirates and Sudan. The study carried out over two years from October 2017 to October 2019.

### Study population and sample size

Seventy patients selected from patients visiting Gynecology clinic from old age groups who had no menstrual cycle for more than one year will be selected as cases.

### Ethical consideration

Permission of this study will be obtained from the local authorities in the area of the study. The objective of the study explained to all individuals participating in this study. An informed written consent obtained from all participants.

### Data collection

Data collected by a designed questionnaire which included demographics and the history of each participant.

### Inclusion criteria

Cases are postmenopausal women presented with bleeding.

### Exclusion criteria

All HPV positive postmenopausal women without bleeding and women in a reproductive age.

### Cytological examination

#### Pap test

The samples collected by using speculum to visualize the cervix and disposable Broom to harvest the transformation zone cellular components. Then the head of the broom soaked repeatedly in the liquid base fixative and the smear prepared using thin prep 5000 machine, fixed in 95% Ethyl alcohol for 15 minutes and stained with Pap stain in Autostainer as follows.

1. 95% Ethanol 1minute
2. Rinse in tap water
3. Harris or Gill Hematoxylin 1-3 minutes (Time vary with selection of hematoxylin solution)
4. Rinse in tap water or Scott's tap water
5. 95% Ethanol 1 minute
6. OG-6 stain for 1.5 minutes.
7. 95% Ethanol 1 minute
8. EA-50 for 2.5 minutes.
9. 95% Ethanol 1 minute, 2 changes
10. 100% Ethanol 1 minute
11. Clear in 2 changes of xylene, 2 minutes each
12. Mount with DPX

#### Microscopical examination and reporting

Olympus microscope used for slides screening looking for any atypical cellular changes, and the slides reported using Bethesda system for reporting cervical cytopathology.

### Immunocytochemical protocol

For overexpression of p53 Immunocytochemical demonstration the Avidin-Biotin (ABC) method was used for immunostaining and applied to Pap smear cytology slides which had been rehydrated through a series of graded alcohols. The immunostaining procedure performed using an automated stainer (Leica IHC Bond Max) the primary antibody used was the mouse monoclonal anti p53 antibody clone DO-7 (1:400, DAKO, Glostrup, Denmark). Then Microwave antigen retrieval performed by placing the slides in 10 mM citrate buffer (pH 6.0) in a pressure cooker (Nordic Ware), and microwaving on high power until the buffer boiled under pressure for 4 minutes. At this point microwaving stopped, and the slides incubated in the pressure cooker for another 20 minutes, removed and rinsed. The antip53 antibody DO-7 recognizes both wild type and mutant p53. Sections counterstained with light haematoxylin. p53 index assessed under a light microscope with magnification (10× and 40× objective).

#### Molecular analysis

Techne 512, UK thermal cycler used to optimize the PCR amplification procedure and the product was analyzed by gel electrophoresis in 1.5% Agarose, and stained with 0.15 % Ethidium bromide and visualized by using UV gel documentation system. (DNA products expected size is 141 bp).

The PCR amplification technique was performed and the test carried out following primers:

F
3'TCCCCCTTGCCGTCCCAA'5
R
3'CTGGTGCAGGGGCCACGC'5

The primers sequences were cited from previous studies and obtained from Macrogen Korea (10F, 254 Beotkkot-ro Geumcheon-gu, Seoul. 08511, Rep of Korea). Each reaction was performed in total volume of 25 ul, containing 5 ul master mix, 2 ul of primer, 5 ul of DNA and 13 ul of distilled water.

Reactions were performed using Techne 512 machine under the following conditions, initial denaturation step at 94 °C for 3 min and then 35 cycles of 94 °C for 30 s, annealing at 60 °C for 30 s, and the elongation step at 72 °C for 30 s. The final elongation step of the PCR was carried out at 72 °C for 5 min.

#### Anti contamination measures

Strict precautions to prevent PCR products' contamination were enforced.

## Statistical Analysis

All information about the study uploaded in a spread sheet as well as obtained results. The data analyzed using 11.5 statistical packages for Social Science programme (SPSS). Frequencies mean chi-square test value then calculated.

## Results

Seventy liquid base samples were collected from ecto and endocervices of postmenopausal women with bleeding and examined cytologically for any cellular changes, Five samples collected from postmenopausals without vaginal bleeding used as a control, they were normal cytologically (NILM) in PAP smear, Negative for IHC stain, and used as control in PCR while the Seventy samples were ranging between reactive cellular changes (Twelve samples) and Normal in cytological examination. While in IHC Twenty seven samples were IHC positive for p53 overexpression. Figures 1-3, ranging between strong positive (Fifteen samples) and weak positive (Twelve samples) (Table 1). Molecular confirmation using Polymerase Chain Reaction (PCR) product was analyzed by gel electrophoresis in 1.5% Agarose, and stained with 0.15 % Ethidium bromide and the product was visualized by using UV gel documentation system. Thirteen samples were p53 expressed with mutational changes. Figures 4 and

5 Data of endometrial and endocervical assessment reports was collected from the HIS, thirteen patients had endometrial thickness ranging between 3 to 12 mm and the endocervices was ranging between normal and inflamed. All samples were HPV negative except one case was Positive for HPV 16 and showed intraepithelial neoplasia in Pap smear and positive IHC and PCR. Thirteen samples out of Twenty seven samples showed strong p53 over expression in PCR and the Fourteen IHC weak positive in addition to one case strong positive showed negative p53 over expression in PCR.

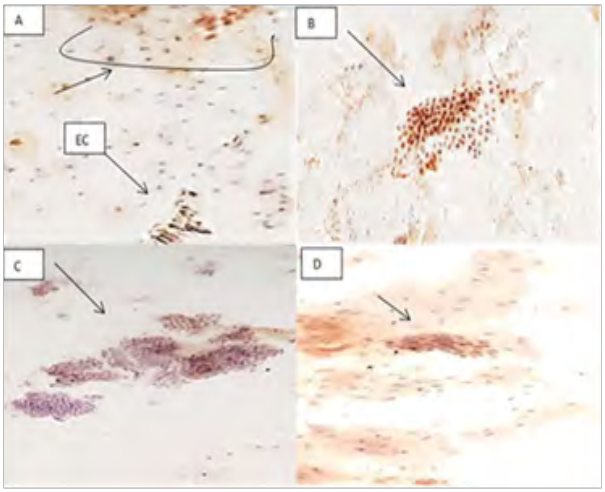


**Figure 1: (A)** Glandular endocervical cells showed positive p53 Immunostain. **(B)** Showed positive p53Immunostain while **(C)** was negative for p53 Immunostain **(D)**.

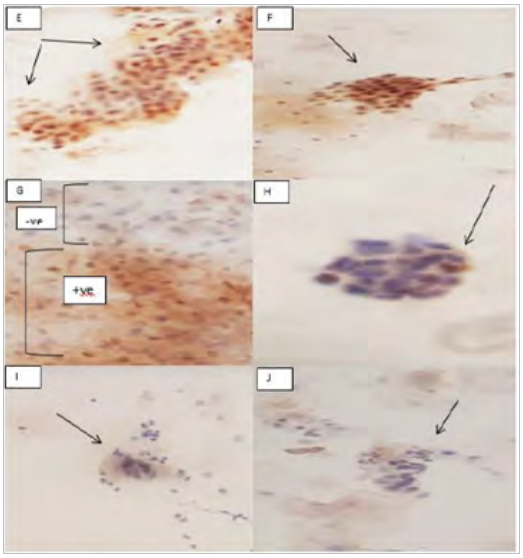


**Figure 2: (E,F)** Showed squamous epithelial cells p53 overexpression in IHC immunostain. **(G)** Showed diphasic picture of positive and negative p53 Immunostain in the same field. **(H)** Endometrial cells showed positive p53 IHC Immunostain. **(I,J)** Endocervical cells showed negative p53 Immunostain.

## Discussion

Knowledge of the oncogenetic pattern of a tumor can explain its natural history and behavior in single cases. Thus, a number of oncogenetic studies of cervical and endometrial cancer have been performed to identify prognostic and predictive markers the p53 tumor suppressor gene is correlated with cervical and endometrial tumor induction and progression, and its significant role as a prognostic marker was found in previous studies in advanced cervical and endometrial tumors.

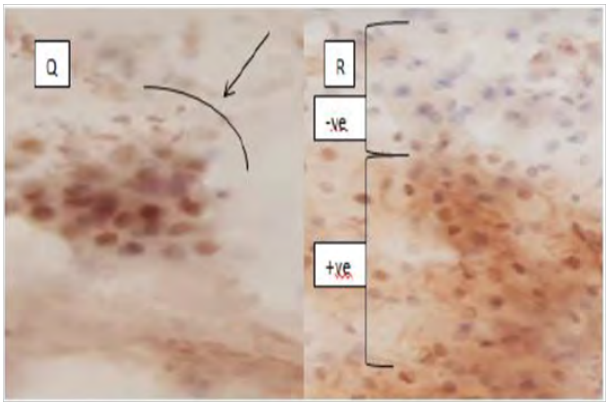


**Figure 3: (Q)** Showed Strong p53 overexpression in endocervical cells, while **(R)** showed positive and negative picture of p53 expression.

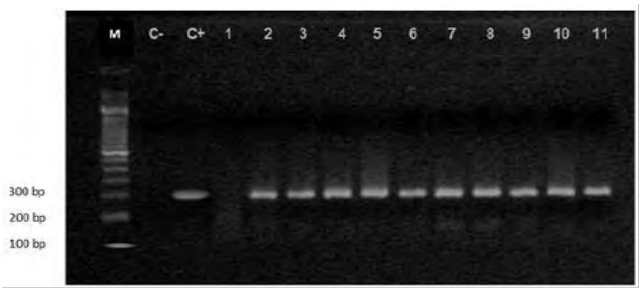


**Figure 4:** Electrophoresis using Agarose gel of p53products. Lane M: 1.5 Kb DNA Ladder. C- Is control negative, C+ is control positive Lanes 2 to 11: p53 PCR products (296 bp). Lane 1: Negative sample.

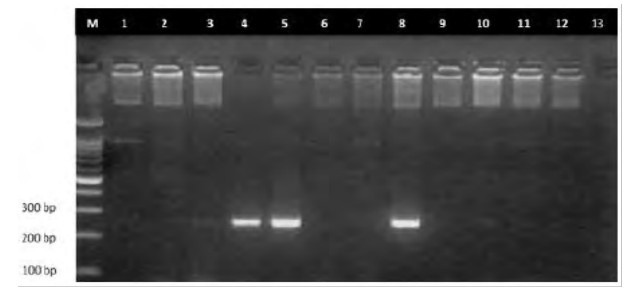


**Figure 5:** Electrophoresis using Agarose gel of p53 PCR product. Lane M: 1.5 kb DNA ladder. Lanes4, 5 and 8 p53 PCR products (296 bp). Lanes 1, 2, 3, 6, 7, 9, 10, 11, 12 and 13) are negative samples.

The aim of the present study was to detect the genetic changes that can lead to uterine cancer. It is important to note that the cohort of patients considered was not consecutive due to the inclusion criteria used. Patients were selected on the basis of Clinical history. This allowed more significance to be assigned to acquire data, as even more emphasis could be placed, in the single case and the association with the risk of mortality.

| Procedure | Positive |
|---|---|
| RCC (Cytomorphology) | 12 |
| IHC 1(Strong) | 15 |
| IHC 2 (Weak) | 12 |
| PCR | 13 |

**Table 1:** Showing the frequency of positive reactions.

Malignant tumors result from an accumulation of genetic alterations that lead to overexpression of oncogenes and inactivation of tumor suppressor genes. The consequences of these changes in cells progressing to malignancy are the loss of negative regulation of cellular proliferation or programmed cell death. Evidence thus far accumulated suggests that normal p53 protein is a negative regulator of cell growth, possibly playing a role in maintaining genomic integrity by arresting cells with DNA damage at the GL/S boundary of the cell cycle. Mutations of the p53 gene are the most common genetic alterations associated with cancers in humans. An important consequence of these various mutations is post translational stabilization of the altered protein, leading to elevated p53 levels in tumor cells. Because of its short half-life, wild type p53 protein is almost undetectable at IHC, and its positive immunoreactivity is considered synonymous with altered protein structure or function due to mutation or viral oncogene involvement. The presence of point mutations in the p53 gene is not the only cause of protein accumulation. Change in p53 half-life may also be caused by exposing normal cells to DNA-damaging agents, such as ultraviolet light. This critical observation has generated renewed interest and led to important new models of p53 protein function. A cell in distress usually reacts by activation of biochemical pathways controlled by the p53 gene. The increase in p53 protein level is associated with an increase in transcription of p53-responsive genes and induction of growth arrest and apoptosis. The precise mechanism by which DNA damage and other stimuli result in p53 stabilization is not yet elucidated, but may involve the action of other gene products. IHC positive and PCR negative results can be explained by the presence of either mutations outside the exons screened or mutant conformers migrating like wild-type strands. However, a non-mutational stabilization of p53 may well be operative. Distressed cells express high levels of wild-type protein, perhaps through interruption of the p53 degradative pathway. Moreover, some p53- dependent genes involved in cell cycle regulation may be altered and may indirectly cause p53 overexpression in cell nuclei. On the other hand, IHC-negative, and positive PCR may result from nonsense point mutations or from deletions, because such alterations lead either to premature cessation of protein synthesis or generation of quite different protein products, making IHC detection impossible or may be related to the presence of point mutations that do not cause p53 protein stabilization

## Conclusion

In the present study, Liquid Base (LBC) PAP smears of 70 women with postmenopausal bleeding with normal Cytomorphological findings ranging between Negative for Intraepithelial Lesion (NILM) and Reactive Cellular Changes (RCC), including 5 samples collected from postmenopausals without bleeding used as a control, were evaluated in this study. Menstruating women or woman in reproductive age were excluded from this study. Expressions of p53 were immunohistochemically examined and the findings confirmed using molecular analysis (PCR). Overexpression of p53 was categorized as weak to strong in more than 18% of the samples. Strong overexpression of p53 was observed in 15 samples (10.5%) and 12 samples (8.4%) showed weak p53 overexpression in Immunohistochemical studies. Molecular analysis (PCR) used to confirm the results and it showed 13 Positive using gel electrophoresis and read under Ultra Violet (UV) light. Twelve out of Fifteen samples which were strongly positive in immunomarker stain and One out of Twelve weak positive samples in immunomarker stain showed positive mutational changes in molecular study. Postmenopausals with bleeding exhibited significantly overexpression of p53.

Overexpression of p53 may be responsible for the high proliferative activity of postmenopausal endometrial intraepithelial neoplasia and /or endocervical intra epithelial neoplasia.

## Recommendations

In this study we found that p53 plays an important role to predict the endometrial or endocervical cancer in postmenopausals with bleeding. Any woman has a vaginal bleeding especially after menopause which may be endometrial or endocervical origin. 10%-20% have genetic aberration and they have the liability to develop endometrial or endocervical cancer has to be under medical care.

## References

1. Ellenson LH, Wu T-C (2004) Focus on endometrial and cervical cancer. J Cancer cell 5: 533-538.
2. Kong A, Johnson N, Kitchener HC, Lawrie TA (2012) Adjuvant radiotherapy for stage I endometrial cancer. Cochrane Database Syst Rev 18: CD003916.
3. National Cancer Institute NCI (2014) National Cancer Institute NCI. US Department of Health and Human Services. Washington, USA.
4. Purdie DM, Green AC (2001) Epidemiology of endometrial cancer. Best Practice & Research Clinical Obstetrics & Gynaecology 15: 341-354.
5. Saso S, Chatterjee J, Georgiou E, Ditri AM, Smith JR, et al. (2011) Endometrial cancer. BMJ Pg no: 343.
6. (IARC &WHO) International Agency for Research on Cancer (2003) World Cancer Report. World Health Organization, IARC Press, Lyon, France.
7. A.V U, G I. POST MENOPAUSAL BLEEDING (PMB) -. Ibom Medical Journal, 6(1). Available from: http://www.ibommedicaljournal.com/m. 2013.
8. Newell S, Overton C (2012) Postmenopausal bleeding should be referred urgently. Practitioner 256: 13-15.
9. Miller DS, Schorge JO (2012) Endometrial cancer. In: Hoffman BL, Schorge JO, Schaffer JI, et al (eds.). Williams Gynecology. McGraw-Hill, New York, USA.
10. McGraw-Hill (2014) Williams Gynecology Endometrial Cancer (2nd edn.) pg no: 818.
11. Ma J, Ledbetter N, Glenn L (2013) Testing women with endometrial cancer for lynch syndrome: should we test all? J Adv Pract Oncol 4: 322-330.
12. O'Mara TA, Glubb DM, Amant F, Annibali D, Ashton K, et al. (2018) Identification of nine new susceptibility loci for endometrial cancer. Nat Commun 9: 3166.
13. Enomoto T, Inoue M, Perantoni AO, Buzard GS, Miki H, et al. (1991) K-ras activation in premalignant and malignant epithelial lesions of the human uterus. Cancer Res 51: 5308-5314.

14. Takata M, Matsui Y (1994) Proliferating Cell Nuclear Antigen (PCNA) and p53 Protein Expression in Bowen's Disease. J Dermatol 21: 947-952.

15. Blagosklonny MV (2002) P53: an ubiquitous target of anticancer drugs. Int J Cancer 98: 161-166.

16. Strachan T, Read A (2018) Human Molecular Genetics (5th edn.), CRC Pres, Pg no: 770.

17. Sherman ME, Bur ME, Kurman RJ (1995) P53 in endometrial cancer and its putative precursors: evidence for diverse pathways of tumorigenesis. Human Pathol 26: 1268-1274.

18. Kounelis S, Kapranos N, Kouri E, Coppola D, Papadaki H, et al. (2000) Immunohistochemical profile of endometrial adenocarcinoma: a study of 61 cases and review of the literature. Mod Path 13: 379-388.

19. Lecce G, Meduri G, Ancelin M, Bergeron C, Perrot-Applanat M (2001) Presence of Estrogen Receptor β in the Human Endometrium through the Cycle: Expression in Glandular, Stromal, and Vascular Cells. Journal of Clinical Endocrinology and Metabolism 86: 1379-1386.

20. American Cancer Society (2017) Breast Cancer Facts & Figures. American Cancer Society, Atlanta, Georgia, USA.

21. Lane D, Levine A (2010) P53 Research: The Past Thirty Years and the Next Thirty Years. Cold Spring Harb Perspect Biol 2: 000893.

22. Ambros RA, Sherman ME, Zahn CM, Bitterman P, Kurman RJ (1995) Endometrial intraepithelial carcinoma: a distinctive lesion specifically associated with tumors displaying serous differentiation. Hum Pathol. 26: 1260-1267.

23. Baergen RN, Warren CD, Isaacson C, Ellenson LH (2001) Early uterine serous carcinoma: clonal origin of extrauterine disease. Int J Gynecol Pathol 20: 214-219.

24. Gilbert SF (2000) Developmental Biology (6th edn). Sinauer Associates, Sunderland, Massachusetts, USA.

25. Lawrence L (2018) Postmenopausal Bleeding Common in Women With Endometrial Cancer, Cancer Net Work, journal of oncology.

26. Soliman PT, Lu KH (2014) Endometrial Hyperplasia, Endometrial Carcinoma, and Sarcoma: Diagnosis and Management. Neoplastic Diseases of the Uterus Pg no: 1-23.

27. Colombo N, Preti E, Landoni F, Carinelli S, Colombo A, et al. (2013) Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology 24: 33-38.

28. SGO Clinical Practice Endometrial Cancer Working Group, Burke WM, Orr J, Leitao M, Salom E, et al. (2014) Endometrial cancer: a review and current management strategies: part I. Gynecol Oncol 134: 385-392.

29. PDQ NIH (2015) Archived from the original.

30. Galaal K, Al Moundhri M, Bryant A, Lopes AD, Lawrie TA (2014) Adjuvant chemotherapy for advanced endometrial cancer. Cochrane Database Syst Rev 15: CD010681.

31. Vale CL, Tierney J, Bull SJ, Symonds PR (2012) Chemotherapy for advanced, recurrent or metastatic endometrial carcinoma. Cochrane Database Syst Rev 8 : 003915.


Journal of Anesthesia & Clinical Care

Journal of Addiction & Addictive Disorders

Advances in Microbiology Research

Advances in Industrial Biotechnology

Journal of Agronomy & Agricultural Science

Journal of AIDS Clinical Research & STDs

Journal of Alcoholism, Drug Abuse & Substance Dependence

Journal of Allergy Disorders & Therapy

Journal of Alternative, Complementary & Integrative Medicine

Journal of Alzheimer's & Neurodegenerative Diseases

Journal of Angiology & Vascular Surgery

Journal of Animal Research & Veterinary Science

Archives of Zoological Studies

Archives of Urology

Journal of Atmospheric & Earth-Sciences

Journal of Aquaculture & Fisheries

Journal of Biotech Research & Biochemistry

Journal of Brain & Neuroscience Research

Journal of Cancer Biology & Treatment

Journal of Cardiology: Study & Research

Journal of Cell Biology & Cell Metabolism

Journal of Clinical Dermatology & Therapy

Journal of Clinical Immunology & Immunotherapy

Journal of Clinical Studies & Medical Case Reports

Journal of Community Medicine & Public Health Care

Current Trends: Medical & Biological Engineering

Journal of Cytology & Tissue Biology

Journal of Dentistry: Oral Health & Cosmesis

Journal of Diabetes & Metabolic Disorders

Journal of Dairy Research & Technology

Journal of Emergency Medicine Trauma & Surgical Care

Journal of Environmental Science: Current Research

Journal of Food Science & Nutrition

Journal of Forensic, Legal & Investigative Sciences

Journal of Gastroenterology & Hepatology Research

Journal of Gerontology & Geriatric Medicine

Journal of Genetics & Genomic Sciences

Journal of Hematology, Blood Transfusion & Disorders

Journal of Human Endocrinology

Journal of Hospice & Palliative Medical Care

Journal of Internal Medicine & Primary Healthcare

Journal of Infectious & Non Infectious Diseases

Journal of Light & Laser: Current Trends

Journal of Modern Chemical Sciences

Journal of Medicine: Study & Research

Journal of Nanotechnology: Nanomedicine & Nanobiotechnology

Journal of Neonatology & Clinical Pediatrics

Journal of Nephrology & Renal Therapy

Journal of Non Invasive Vascular Investigation

Journal of Nuclear Medicine, Radiology & Radiation Therapy

Journal of Obesity & Weight Loss

Journal of Orthopedic Research & Physiotherapy

Journal of Otolaryngology, Head & Neck Surgery

Journal of Protein Research & Bioinformatics

Journal of Pathology Clinical & Medical Research

Journal of Pharmacology, Pharmaceutics & Pharmacovigilance

Journal of Physical Medicine, Rehabilitation & Disabilities

Journal of Plant Science: Current Research

Journal of Psychiatry, Depression & Anxiety

Journal of Pulmonary Medicine & Respiratory Research

Journal of Practical & Professional Nursing

Journal of Reproductive Medicine, Gynaecology & Obstetrics

Journal of Stem Cells Research, Development & Therapy

Journal of Surgery: Current Trends & Innovations

Journal of Toxicology: Current Research

Journal of Translational Science and Research

Trends in Anatomy & Physiology

Journal of Vaccines Research & Vaccination

Journal of Virology & Antivirals

Archives of Surgery and Surgical Education

Sports Medicine and Injury Care Journal

International Journal of Case Reports and Therapeutic Studies