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Study of etiology and histopathological evaluation in women with post-menopausal bleeding: A prospective study

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Abstract
Background: Postmenopausal bleeding (PMB) is a common clinical problem. Patients with PMB have 10%–15% chance of having endometrial carcinoma and therefore the diagnostic workup is aimed at excluding malignancy. Transvaginal sonography (TVS) is used as a first step in the diagnostic workup, but different authors have come to different conclusions assessing the accuracy of TVS for excluding endometrial carcinoma. Diagnostic procedures obtaining material for histological assessment (e.g., dilatation and curettage, hysteroscopy, and endometrial biopsy) can be more accurate but are also more invasive. The best diagnostic strategy for diagnosing endometrial carcinoma in patients with PMB still remains controversial. Future research should be focussed on achieving a higher accuracy of different diagnostic strategies.
Objective: Aim of this study is to evaluate women with post-menopausal bleeding in terms of etiology, risk factor and most common cause of post-menopausal bleeding.
Method: Prospective study of 150 patients over 2years with post-menopausal bleeding evaluated by TVS, endometrial biopsy, per speculum and cervical biopsy for mass.
Univariate and multivariate logistic regression identified factors associated with risks of endometrial hyperplasia.
Result
1. In this study mean age at presentation was 59.7yr
2. Mean ET was 6.7mm
3. HPE was s/o atrophic endometrium 2.3%, endometrial hyperplasia 6.4%, ca endometrium12.2%, proliferative endometrium 9.4%, endometrial polyp 2.9%. ca cervix 39.5%.
4. Using multivariate logistic regression, we found that ET, recurrent heavy menstrual bleeding, diabetes mellitus, obesity were better predictiors of endometrial cancer.
5. Most common cause of post-menopausal bleeding in India is ca cervix with histopathology of squamous cell cancer being more common.

Conclusion: The postmenopausal bleeding is an important symptom and requires careful and timely assessment to eliminate the possibility of malignancy as soon as possible.

Keywords: Endometrial hyperplasia, endometrial cancer, post-menopausal bleeding, endometrial thickness, CA cervix

Introduction

Menopause is defined as permanent cessation of menstruation caused by ovarian failure with the average age at menopause being 52 yr, ranging from 40-58yr [1].

Post-menopausal bleeding is defined as uterine bleeding after permanent cessation of menstruation resulting from loss of ovarian follicular activity, the time interval defined is approximately of 12 months however because an ovulatory cycles with episodes of multi month amenorrhea frequently precedes before menopause, no consensus exist regarding the appropriate interval of amenorrhea before an episode of bleeding that allows for the definition of post-menopausal bleeding. Post-menopausal bleeding is thus a retrospective diagnosis as the time of final menstrual period followed by 12 month of amenorrhea [1].

Bleeding can be spontaneous or related to use of exogenous estrogen (HRT). As carcinoma of the genital tract is one of the most important causes of post-menopausal bleeding a thorough evaluation is of utmost important to ensure the cause as a benign pathology.

90%of the patients with endometrial cancer present with post-menopausal bleeding. 10% patients with post-menopausal bleeding have CA endometrium, hence a bleeding episode should

not be neglected. Formerly the gold standard investigation was institution based dilatation and curettage but now office based endometrial sampling techniques exist. Post-menopausal bleeding accounts for 5%of the office gynecological presentation with a strong suspicion towards malignancy [2], benign pathologies account for a major fraction among the causes. Endometrial atrophy accounts for 60-80%, endometrial or cervical polyp accounting for 2-12%, exogenous estrogen about 15-25% and carcinoma endometrium accounting for 10 % [1]. This study aims at studying the significance of post-menopausal bleeding in terms of risk factors, incidence of malignancy, most common aetiology and histopathological evaluation.

The GLOBOCAN 2020 data of no of new cases of various cancers in world [9].

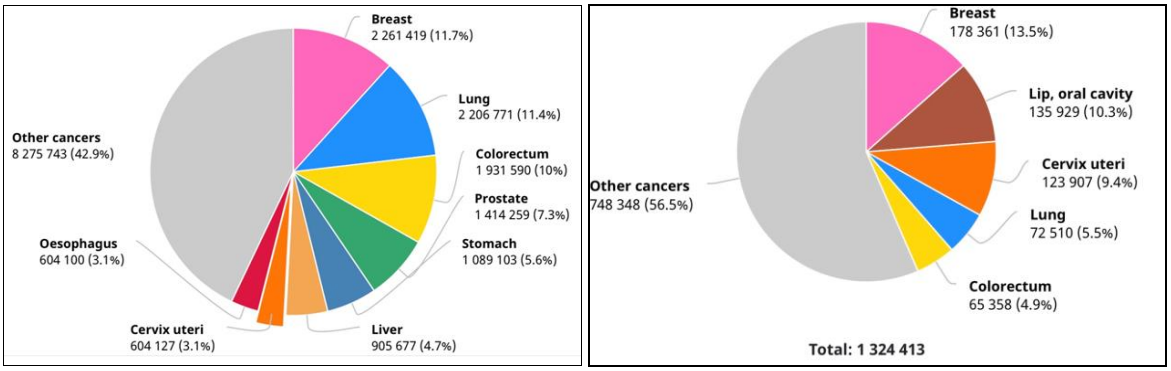


Fig 1: GLOBOCAN 2020 data of no of deaths from various cancers [9].

Data in India 2020 of cancer study [9]

Important causes for evaluation in case of post-menopausal bleeding include.

Endometrial Hyperplasia

Defined as proliferation of glands of irregular shape and size with an increase in gland to stroma ratio approximately 15% of the women presenting with postmenopausal bleeding will be diagnosed with endometrial hyperplasia. Mostly occurs in the age group of early 50's to 60's.

It is associated to exposure of unopposed oestrogen that can be from exogenous or endogenous sources. Endogenous sources include increased estrogen secondary to; Peripheral conversion of in androstenedione adipose tissue especially in obese women. Estrogen secreting ovarian tumors such as granulosa cell tumors and ovarian thecomas. Exogenous sources can be HRT AND SERMS.

Endometrial carcinoma is the m/c malignancy in the US (Ca cervix being (m/c) in India). Fourth m/c cancer and leading cause of death. Increasingly virulent with advancing age hands occurs primary in postmenopausal age group.

Risk factors

1. Nulliparity; has two to three times the risk as compared to parous women.
2. Infertility and H/O irregular menses due to an ovulatory cycle increases the risk. Pregnancy is a state with high progesterone. Anovulation is a condition without sufficient progesterone. Thus, leading to increased risk. Menopause occurring at the age of > 52 years increases risk by 2.44 fold as compared two doors before 49 years. As a result of prolonged exposure uterus to progesterone deficient environment.
3. Overweight/obese: risk is increased three times in women who are 21 to 50 pounds and 10 times in > 50 pounds. This is due to aromatisation of adipose tissue. Obesity is leading to increase rates of insulin resistance thus causing metabolic syndrome.
4. A history of polycystic ovary syndrome increases the risk mainly due to anovulatory cycles.
5. Menopausal oestrogen therapy prolonged and without progesterone* and also use of Tamoxifen (SERM) increases risk.
6. Diabetes mellitus and hypertension are also associated comorbidities that increase the risk.
7. Genetic influences germline mutation in MMR genes MLH1, MSH2, MSH6 have 40 – 60% life time risk.
8. Women with Lynch syndrome II are prone to develop endometrial cancer.

Ca cervix

Cervical cancer is a potentially preventable disease and the most common malignancy in developing countries. Persistence of the infection with human papilloma virus and subsequent dysplasia lead to cervical cancer. The incidence is declining in developed countries due to well established screening programs. While in developed countries the lack of effective screening and low utilisation of the services are the common factors responsible for high incidence and death.

In developing countries it is the second most common cause of cancer related deaths. It is called a preventable disease as it has a long pre invasive state which can be detected by screening methods. It has a bimodal age of presentation *i.e* one peak at 35-39yr and second from 60-64yr. Risk factors associated are:

Young age at first intercourse
Multiple sexual partners
Smoking
High parity
Low socio economic status

Chronic inflammatory disease

The initiating event is infection with HPV with herpes virus and chlamydia trachoma's acting as cofactors. HPV is the causative agent for both squamous and adenocarcinoma of cervix. The mechanism of carcinogenesis is as follows. The high risk HPV virus include HPV 16 and 18 are found in 70% of the cancer patients.

Symptoms

Vaginal bleeding is the most common presenting symptom. It can be in the form of post coital bleeding, irregular bleeding or post-menopausal bleeding.

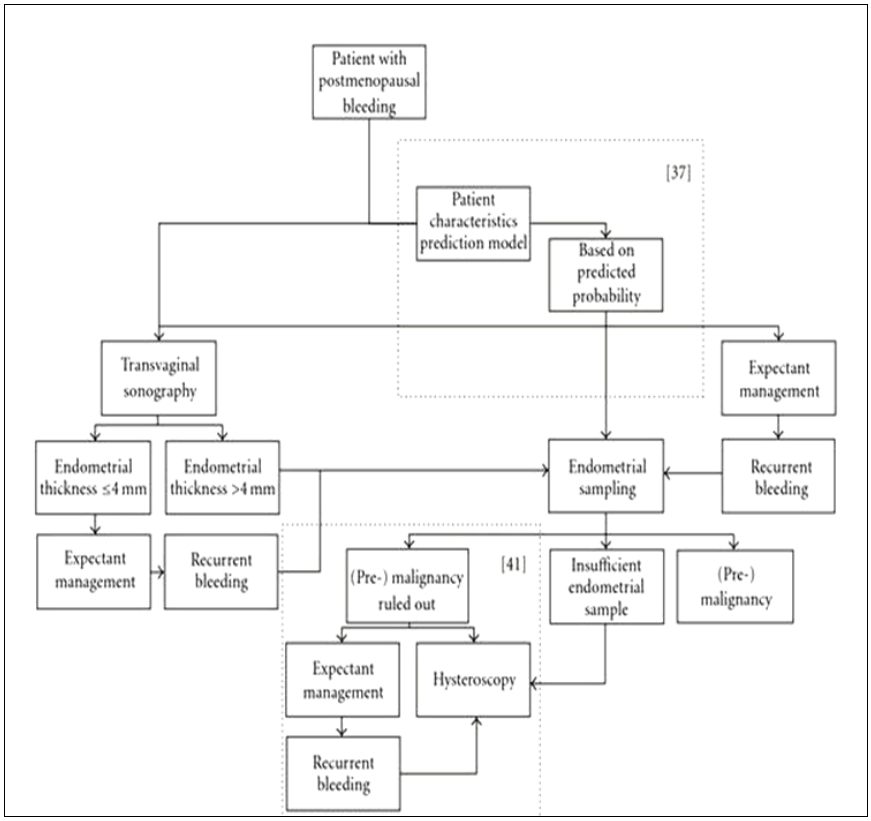


Fig 2: Diagnostic algorithm for evaluation of women with post-menopausal bleeding

Thus, Postmenopausal bleeding (PMB) requires complete assessment in order to ensure the absence of malignancy and to identify and treat high risk patients such as those with endometrial hyperplasia. The aim of the present study was to investigate the clinical significance and endometrial pathology in patients with postmenopausal bleeding.

Aim and objective of the study

1. To study the clinical profile of post-menopausal women presenting with bleeding per vaginum in terms of age, parity, duration of post-menopausal bleeding, body mass index, associated medical disorders and time since menopause.
2. To study the etiological factors of post-menopausal bleeding and establish various causes of post-menopausal bleeding through histopathological evaluation.
3. To determine the incidence of aetiologies linked with post-menopausal bleeding.

Method

This is a prospective observational study for a period of 24 month including 18 month of data collection and 6month of compilation of the results. The study will include the indoor as well as outdoor patients of the department of obstetrics and gynaecology at the tertiary care centre of GMCH, nagpur.

Study population will include. All post-menopausal women presenting with the complaints of bleeding pervaginum with their last menstrual period 1 year back. Women with blood tinged mucoid or watery vaginal discharge.

Exclusion criteria will be: Premature menopause

Surgical menopause

Radiation and chemotherapy induced menopause nticoagulant therapy

Coagulation disorder

Injuries to genital tract

Representation of the study pattern

The present study was done after approval of Institutional Ethics Board and after taking informed consent from women.

1. Women presenting with post-menopausal bleeding
2. Detailed history taking, age at menopause, time since menopause, body mass index, any unscheduled vaginal bleeding with use of MHT, presence of hypertension and diabetes, single episode or recurrent episodes of vaginal bleeding. Recurrent episodes were defined as any bleeding episode lasting 7 or more days or two or more separate bleeding events within the past 12 months.
3. Examination including the BMI done.
4. USG pelvis done with endometrial thickness
5. Endometrial sampling done either after admission or by pipelle biopsy.
6. If visible growth available then sample sent for histopathological examination women with ET equal to or >5 mm were admitted for dilatation and curettage (D and C) under anaesthesia to yield sufficient tissue for histological diagnosis. The histopathological examinations were performed by pathologists and the reports of the curettage were reviewed for all patients.

Statistical analysis

Data were summarized using standard descriptive methods, frequency and percentages for categorical variables, and mean and SD or median and range for continuous variables. Comparisons between categorical variables were tested by the use of contingency tables and by the calculation of the Chi-square test. Comparisons between normally distributed continuous variables and categorical variables were performed using Student's *t*-test and analysis of variance, whereas the nonparametric Mann–Whitney and Kruskal–Wallis tests were

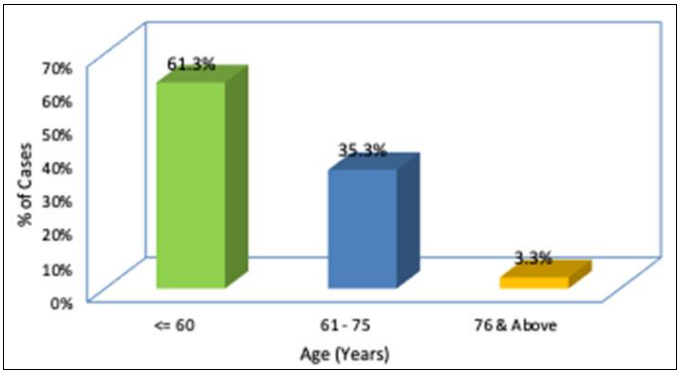
used for asymmetric continuous variables. All calculated *P* values were 2 sided and $P < 0.05$ were considered statistically significant.

Sample size
With absolute precision level 8%
And confidential interval 95%
The required sample size is 150.

Results and Observation
This study to assess the etiology and histopathological evaluation of women with post-menopausal bleeding was carried out among 150 women and the results are as follows:

Table 1: Shows the mean age at presentation of women with post-menopausal bleeding.

Age (Years)	No. of Subjects	Percentage
<= 60	92	61.3%
61 - 75	53	35.3%
76 & Above	5	3.3%
Total	150	



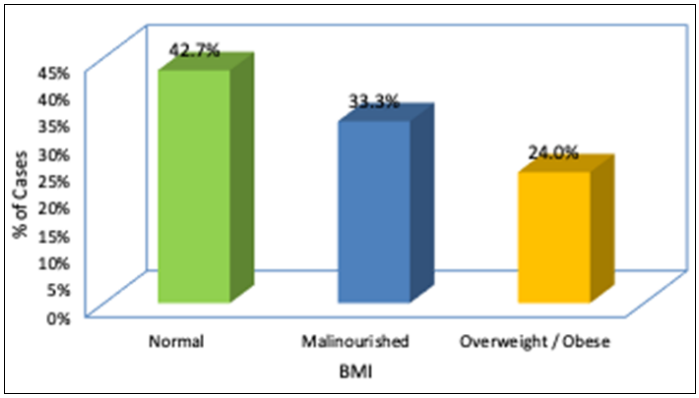
Graph 1: Age distribution

Age group	Benign	Malignant	Total
45-50yr	14	7	24
51-55yr	11	19	36
56-60yr	8	15	32
>60yr	5	52	58
	P value	<0.001	

Most patients presenting at 6th decade with maximum having malignant aetiologies. When talking about the benign pathologies presentation is between 45-55yr and among the malignant patients present at two age groups between 50-55yr and then between 60-70 yr. The mean age at presentation being <60yr.

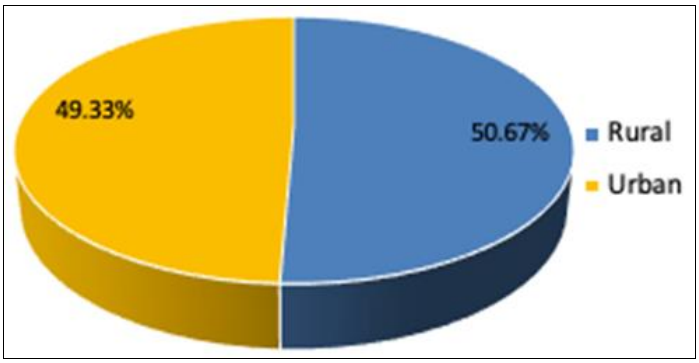
Table 2: Demographic characteristics

Comorbidity	No. of Subjects	Percentage	Value
Any Comorbidity	110	73.3%	0.068
Obesity	35	23.3%	0.172
HTN	39	26.0%	0.018
DM	13	8.7%	0.412
Contraceptive Use	12	8.0%	0.001
Smoking / Tobaco	47	31.3%	<.001
Low Socioeconomic Status	27	18.0%	0.002
High Socioeconomic Status	2	1.3%	0.044
Hypothyrodism	8	5.3%	0.797
Family History	4	2.7%	0.152
HTN + Obesity	27	18.0%	0.024
DM + Obesity	9	6.0%	0.466
HTN + DM	5	3.3%	0.748



Graph 2: BMI

Residential Status	No. of Subjects	Percentage
Rural	76	50.7%
Urban	74	49.3%
Total	150	

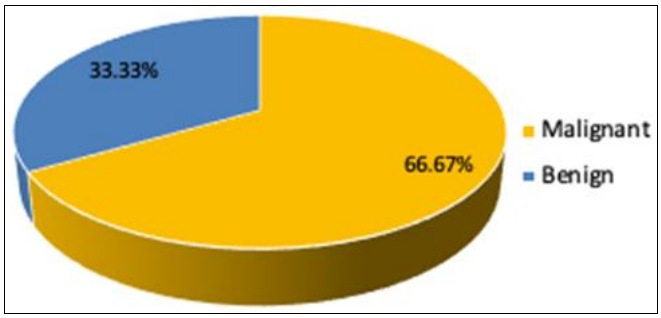


Graph 3: Residential status

Following table presents the association of various demographic features with aetiologies of post-menopausal bleeding. When talking about the associated medical conditions obesity, hypertension and diabetes were most often associated with ca endometrium and few pre- malignant conditions like endometrial hyperplasia. The patients having ca cervix often presented at late stages and were more cachexic. About 10%endometrial cancers have hereditary origin. However, in the present study only 3 cases had significant family history ie 2%. This could be due to few shortcomings like: smaller sample size
The population is less educated and had very little knowledge regarding the cancerous condition or if their first degree relatives had any such condition.
HRT does have a significant impact on endometrium and causes an increased risk of endometrial cancer. However our study does not document any user of HRT due to less knowledge and less preference or usage by Indian women.
Many (44 0f 150) cases of ca cervix had history of tobacco consumption. These were mostly females of low education status and lower socioeconomic classes with habit of gutka consumption. Out of all these few cases were reluctant to give any significant history regarding smoking or tobacco consumption.
On coming to multiple sexual partners, this is still an under evaluated risk factor in this study as very few were ready to give us history if their husband had another partner or if they did so. Also, single unmarried or female separated from their husbands is yet not very prevalent in our country.

Table 3: Aetiologies related to post-menopausal bleeding

Histopathological examination	No. of cases	Percentage
Ca endometrium		
A) Papillary	4	2.3
B) Endometriod	15	8.8
C) Others	2	1.1
Endometrial hyperplasia		
1)without atypia	7	4.1
2)with atypia	4	2.3
Proliferative phase	16	9.4
Secretory phase	2	1.1
Atrophic endometrium	4	2.3
Endometrial polyp	5	2.9
Ca cervix squamous cell		
A) Keratinising	7	4.1
B) Non keratinising	7	4.1
C) Moderately differentiated	45	26.6
D) Other types	8	4.7
Inconclusive	4	2.3
Total	130	76



Graph 1: Final Diagnosis

The current study shows that the maximum cases of post-menopausal bleeding had a malignant etiology with 66% and benign with 33.33%. Ca cervix constitutes the most common cause among the malignancies with moderately differentiated squamous being the most common histopathological finding. While CA endometrium with endometriosis type constitutes the common histological finding among the malignancies.

Discussion

Here, we will try to answer the questions posed as our research questions and also try to see how far is the primary hypothesis put forward by us true.

Time since menopause and bleeding

Study	Year	Result (mean years)
W H lee *et al*., singapore	1991-1992	7.5
Wondwossen *et al*., ethiopia	1994-1997	7.7
Rita D *et al*, Karnataka	2013	1-3yr
DEEPTI *et al*., Gujarat	2017	7.7
Jasmina begum *et al*., Puducherry	2019-2020	7.95
Current study	2019-2020	1-10yr

Study done by Bruchim *et al*. reported on the combination of age since menopause and endometrial thickness in predicting the risk of pre malignancy or malignancy. They showed a linear relationship between the age and risk of malignancy. While another study done by van door *et al* in 2007 showed that the relation of malignancy with age was not linear and increased every year.

The explanation to this could be that in the initial years the bleeding could be due temporary ovarian flare up and other non-malignant causes. Hence, with time endometrium becomes atrophic and causes of bleeding are more due to malignancies. So, the time since menopause should be stonger predictor than age under 55yr.

Results of our present study showed that the mean age was about 59yr and the incidence of malignancy peaked between age 60-75yr.

Considering the low education status of women and with maximum population belonging to rural areas the data provided on age is less reliable. Indian rural population is less concerned with their age or the age of menopause. The age provided is only an estimate. Also here the time since menopause is a better predictor of the aetiology especially ca endometrium.

Also, according to a study in UK from 2006-2009 by nikolaos *et al*. showed that the incidence of malignancy of endometrium peaked at 60-64yr. The incidence of cervical cancer was very low (only 2 cases).

Ilan brachium study in Israel suggested that Time since menopause and the ET together define the cutoff points for diagnostic biopsy of tissue sample for endometrial carcinoma ie when using cutoff 6mm for women experiencing menopause 5-15yr prior and for women with ET 5mm undergoing menopause 15yr or more approx 60% should avoid invasive procedures. Also the probability of cancer increases with time since menopause.

Very few studies have studied the relation between the age at presentation with the causes of vaginal bleeding. Most studies have not considered age as a parameter in prediction of the risk of cancer and other factors like BMI, hypertension, diabetes, episodes of bleeding and ET as even more significant in assessing the risk.

Table 2: Mean age at presentation

Statistics			
		Age (Years)	**Bleeding Duration (Months)**
N	Valid	150	149
	Missing	0	1
Mean		59.77	3.9410
Std. Error of Mean		0.679	0.57604
Median		58.50	2.0000
Mode		55	1.00
Std. Deviation		8.314	7.03152
Range		42	71.93
Minimum		45	0.07
Maximum		87	72.00

Study	Year	Mean age
Shahnaz rahman *et al*.	2004	57.74
W H lee *et al*.	1992	58.1
Min Kyoung Kim *et al*.	2005-2014	62.1
Jasmina begum	2019	57.17
Current study	2019-2020	59.77

Risk factors

Endometrial cancer

Studies by Nirupama *et al*. showed that obesity, hypertension and diabetes were the most commonly associated with carcinoma of endometrium with association of 45%, 36% and 13% among each group. The present study showed a relation of:

	Final Diagnosis		P Value	Odds Ratio
	Malignant	Benign		
Any Comorbidity	78	32	0.068	1.994
Obesity	20	15	0.172	0.583
HTN	20	19	0.018	0.408
DM	10	3	0.412	1.741
Contraceptive Use	3	9	0.001	0.141
Smoking / Tobaco	45	2	<0.001	19.636
Low Socioeconomic Status	25	2	0.002	8
High Socioeconomic Status	0	2	0.044	0
Hypothyrodism	5	3	0.797	0.825
Family History	4	0	0.152	Not Applicable
HTN + Obesity	13	14	0.024	0.384
DM + Obesity	7	2	0.466	1.807
HTN + DM	3	2	0.748	0.742

Study by Astha Abuja *et al*. showed hypertension and diabetes were most commonly associated with ca endometrium and most had BMI>29kg/m2.

In a study, cervical cancer screening and assessment of utilization of sources by Anikwe *et al*. in 2012 showed that incidence of presentation was 46% in nullipara and 49% with parity between 1-4. Age between 51-60yr accounted for 2%.

Study by Azhar Mouse *et al*. showed that most common age for endometrial cancer was in the 5th decade while foe ca cervix it was between 6th-7th decade smoking/ tobacco consumption was more associated with ca cervix while exogenous estrogen and HRT were more associated with endometrial cancers.

Obesity, diabetes and hypertension were more significant with endometrial cancers.

Study done by Jasmin began *et al*. in Odisha also showed the significant predictive variables associated with endometrial neoplasia are ET, recurrent episodes of bleeding, and diabetes.

Study	Year	ODDS ratio of DM	Hypertension or of BMI Obese	
N Burbos *et al*.	2009	1.92	38%	1.03
Hussain hassan *et al*.	2002	18%	80%	
R.sindhuri *et al*, puduvherry	2016			0.44
Jasmina begum *et al*., Odisha	2015-2016	8.03	0.67	2.30
Maryam ghanbari *et al*.	2012-2016			3.9
Current study	2019-2020	1.74	0.40	0.58

Comparison between endometrial thickness with aetiology

In a study conducted in Israel in 2003 found that with a cutoff of 6mm of endometrial thickness the sensitivity and specificity was 96% and 53% in diagnosis of endometrial cancer. While taking a cut off of 5mm, the sensitivity and specificity is 100% and 33% for detecting endometrial cancer. According to the study the frequency of endometrial cancer increased from 0% to 19% when thickness increased from 5mm to 9mm. This study showed a significant relation between time since menopause, endometrial thickness and endometrial carcinoma. The risk of endometrial carcinoma increases with the time elapsed since menopause.

Endometrial thickness	Endometrial cancer	Premalignant lesion
<5mm	9	2
5-8mm	12	
>8mm	15	2

In the present study

Study	Year	Mean ET
Kadakola *et al*.,		8.84
R. Sindhuri *et al*.	2016	11.13
Parvathavarthini *et al*. Karpaga institute	2018	20mm in endometrial cancer, 14.3mm in hyperplasia
Jasmina begum *et al*. odisha	2016	11mm
Valentina tofiloska *et al*.,	2019	10.8mm
Present study	2020	6.8mm

Table 3: Comparison with various studies

Study	Benign (%)	Malignant (%)	Ca cervix (%)	Ca endometrium (%)
Lee *et al*. Singapore, 1995	75	25	12.9	11
Wondwossen *et al*., Ethiopia, 2001	39	61	51	6.5
Shahnaz Rehman *et al*. Bangladesh, Dhaka, 2004	32	18	12	6
Deepti Sharma *et al*, Gujarat 2009-2010	76	24	12.6	6
Rita D *et al*. Karnataka, 2013-2014	94	6		6
Kim *et al*. 2014, Korea	87	13	6.9	5.7
Dr Kavitha, Tamil Nadu 2012-2015	70.2	20.2		13.5
Sreelatha *et al*., Bangalore 2017	54	14	10	4
M . Pavani *et al*., Hydrabad, 2016-2017	44	18	14	4
Present study at GMCH nagpur, 2019-2020	34.13	55.68	41.3	12.5

The current study had results similar to the Ethiopian study done in 2001 showing more number of malignant cases than the benign ones. Yet among the malignant cause Ca cervix remains the most common. The main reason attributed is lack of screening and late reporting of symptoms by the women.

Conclusion

Improved medical practices and increased life span among

women is resulting in more women in the post-menopausal age group and more exposure to hormones.

1. The present study revealed that the incidence of post-menopausal bleeding was more common from 5th to 7th decade of life.
2. The study showed a significant risk of carcinoma among the women with risk factors like obesity, hypertension and diabetes attributable to the unopposed oestrogen concentration.
3. The most common cause of postmenopausal bleeding in the present study was ca cervix and ca endometrium.
4. The study at this tertiary care centre had different results compared with other studies with malignancies forming the maximum percentage of cases. Benign were fewer in number and atrophic vaginitis which was the most common cause in other studies did not stand its place in the present study.

Ca endometrium and Ca cervix were the most common causes evaluated and patients presented at late stages with bladder involvement and metastasis.

The reason for this could be:

1. Pooling of more advanced cases in the tertiary care centre for management.
2. Lack of awareness, education and screening programmers thus ignoring the symptom at an early stage.
3. Factors like obesity, hypertension, recurrent bleeding episodes could be use as parameters for prediction and high susceptibility of cancer and such women should undergo repeated evaluations.
4. One factor which forms a very important risk factor ie hormone replacement therapy in the western countries has a very limited role in Indian population. People are still unaware about this modality and its uses. Hence its use is limited and so are the side effects.
5. Radiological parameters like endometrial thickness, irregularity and difference in the echogenecity of the endometrium in comparison to the surrounding myometrium were important parameters in prediction of cancer. Thus, the first step in evaluation of a patient with post-menopausal bleeding is TVS. This helps us in determining the need to expose the patient to invasive procedure or not.
6. However, recurrent bleeding despite thin endometrium and presence of cancer in patients with a thin endometrium mandates the need to assess the risk factors and then plan for the invasive procedure and fractional curettage.
7. Post menopausal bleeding is a symptom of varied etiology and requires careful examination and accurate diagnosis. Priority should be given towards more screening services at different health care levels to promote early diagnosis along with increasing awareness and educating the women regarding the dangers of ignoring such symptoms
8. Training of the health care workers at each level even at simple techniques of visualisation with acetic acid and visualisation in lugol's iodine can help in identifying the cases earlier and reduction in mortality.
9. Social media, campaigns, ASHA workers can play a very significant role in alerting the women regarding the need to take these symptoms seriously and leave behind all the shyness and embarrassment faced by the women for their own betterment.
10. Government programmes to include the HPV vaccine under the immunisation programme can also help in curbing this problem to a great extent.
11. Ca cervix being a preventable disease we should not miss the opportunities to screen it. Education and utilisation of this opportunistic screening protocol at a larger scale can help preventing the death and misery associated with cancer.

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