The Impact of Hormonal Contraceptives on Breast Cancer Pathology

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Abstract

This retrospective case series study, using data obtained through questionnaires and histopathological diagnoses from 656 patients enrolled in the Department of Defense (DoD) Clinical Breast Care Project (CBCP), evaluated associations between hormonal contraceptive use and breast cancer pathology including benign breast pathologies. Three combination hormonal contraceptive agents (COCs) Lo Ovral (LO), Ortho Novum (ON), and Ortho Tri-Cyclen (OTC) were evaluated as they represented the most commonly used hormonal contraceptives in our cohort. The results of this study suggest that the ever use of LO + ON + OTC does not influence the overall incidence of benign breast condition or malignant disease compared to other COCs; however, patients that have used OTC had an association with a diagnosis of benign or luminal A pathologies whereas ON was associated with a diagnosis of benign and DCIS; LO showed no association with any diagnosis—benign or malignant. Patients that have used LO or ON were more likely to be diagnosed with breast cancer at age ≥ 40 years whereas patients that had ever used OTC were likely to be diagnosed before the age of 40. Caucasians were less likely to have used OTC and more likely to have used ON; however, use of either hormonal agent positively correlated with premenopausal status at diagnosis and having a benign condition. Age at diagnosis, ethnicity, BMI, family history, menstruation status, and duration of use were all independent predictors of different histopathological subtypes. We conclude that patient-specific variables should be considered when deciding on which type of hormonal contraceptive to use to minimize the risk of developing breast cancer or a breast-related pathology.

Introduction

Breast cancer estimates for 2017 in the USA show an estimated 252,710 new cases of invasive breast cancer, 63,410 new cases of breast carcinoma in situ, and 40,610 breast cancer deaths [1]. This makes breast cancer the most common form of cancer and second leading cause of cancer-related deaths among women [1, 2].

Therefore, it is imperative to identify and understand relevant risk factors associated with breast cancer development in order to decrease the risk of its development and mortality.

To date, various patient factors have been identified for their correlation with breast cancer, and these include family history, age, ethnicity, breast density, age at menarche, contraceptive use, diet, obesity, and physical inactivity among others [3–11]. Given that many forms of breast cancer are hormonally dependent, the use of hormonal contraceptives has therefore gained much attention [7, 12]. Hormonal contraceptives are the most widely prescribed form of birth control and represent 13% of the 140 million users worldwide [13, 14]. The Center for Disease Control reported that between 2011 and 2013, 61.7% of women aged 15–44 were using some form of contraception, noting that 23.7% of those were hormonal in nature [15]. There are two general classes of hormonal contraceptives available: combination contraceptives (estrogen plus progestogen) and progestin only contraceptives. The mechanism of action of hormonal contraceptives is to prevent pregnancy by suppressing ovulation through feedback inhibition on the hypothalamic-pituitary axis [16]. In addition to contraception, many formulations also offer non-

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contraceptive benefits, including decreased incidence of anemia, decreased dysfunctional bleeding, decreased dysmenorrhea, reduced bone mineral density loss, and treatment of vasomotor symptoms [17–21].

Controversy exists as to whether the ever use of hormonal contraceptives increases the risk of breast cancer development [9, 22–37]. Currently, all hormonal contraceptives carry warnings and precautions for increased breast cancer risk. In addition, the International Agency for Research on Cancer (ISDA) has classified exogenous estrogen and progestogen as a group I carcinogen due to estrogen and progestin's mitogenic actions and estrogen's carcinogenic metabolites [12, 38].

Hormonal contraceptive-associated breast cancer appears to be dependent upon patient factors, formulations, and duration of use [3–11, 23, 39]. According to the North America Menopause Society, breast cancer incidence increased with estrogen/progestin therapy use beyond 3–5 years in menopausal women; breast cancer mortality was higher in women assigned to estrogen-progestogen therapy versus placebo during the first 5 years of menopause; and these breast cancer risks declined 3 years after cessation of therapy [39]. Several studies also suggest that there is an increased risk of breast cancer when using therapies that contain combination estrogens and progestins [13, 40] and this may be dependent upon the type of estrogen [40] and progestin [12, 38, 41–46]. Even though myriad studies have reported on the potential association of breast cancer risk and molecular subtypes with hormonal contraceptive use, the findings are still not clear [34, 47–52]. Also, many of these studies did not assess relationships between the development of benign condition or malignant neoplasms following the ever use of specific hormonal contraceptive formulations and patient risk factors. In the present study, we determined the association of specific hormonal contraceptive formulations, specifically Lo Ovral (LO), Ortho Tri-Cyclen (OTC), and Ortho Novum (ON), with breast cancer in general and with the development of histopathological subtypes. Furthermore, we assessed documented risk/protection factors utilizing multinomial logistic regression models to evaluate the likelihood that the ever use of a specific hormonal contraceptive agent combined with specific patient factors can lead to the development of specific breast pathologies—both benign and malignant.

Methods

Ethics Statement Appropriate IRB approval was obtained for the Clinical Breast Care Project (CBCP) from the study sites at the Walter Reed National Military Medical Center and Windber Research Institute/Windber Medical Center. IRB approval was also obtained from Duquesne University School of Pharmacy and Division of Pharmaceutical, Administrative and Social Sciences. All study subjects were duly consented

patients enrolled into the Clinical Breast Care Project (CBCP) protocols at the study sites. They were assigned unique research numbers and all samples (blood and solid tissue) were also appropriately coded to maintain patient confidentiality.

Subject Selection This study drew from patients enrolled in the CBCP protocols between December 2000 and September 2015. Patients were stratified based on their use of hormonal contraceptive agents, pathology (benign, atypical, in situ, invasive, and pathological subtypes [luminal A, luminal B, HER2, triple negative-TN]), demography, and medical/family history. Questionnaires were utilized for the collection of all required information and pathological data was documented by a licensed pathologist. Using these questionnaires, patients were deemed unqualified if they lacked information on the formulation name or lacked consistent hormonal contraceptive prescription identification. Patients lacking complete pathology data for the breast cancer subtype were also disqualified. From a total of 4299 patients with information on contraceptive utilization, only 656 had adequate hormonal contraceptive and pathology data to qualify for the study. Patient selection for study inclusion is illustrated in Fig. 1.

Data Query All patient information associated with the questionnaire and pathology diagnosis was then queried within the CBCP Data Warehouse for Translational Research and the results exported to Excel spreadsheets for data analysis.

Patient Variables This study includes patients with benign breast conditions, carcinoma in situ, specifically ductal carcinoma in situ (DCIS) and invasive breast cancer. Immunohistochemistry was performed for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), and Ki-67 on all of the invasive BC cases by a Clinical Laboratory Improvement Association (CLIA) certified laboratory. A tumor was considered to be positive for (1) ER or PR when $\geq 5\%$ of the cells were positive, (2) HER2 + if IHC staining intensity was a 3+ or fluorescence in situ hybridization (FISH) indicated a ratio of ≥ 2.2 for copies of the HER2 gene to the centromere of chromosome 17, (3) Ki-67 + when $\geq 15\%$ of the cells were positive. Determination of the histopathological subtype is summarized in Table 1 and is the response variable in this study [53].

Statistics To determine the likelihood of the presence of an observed variable within and between the selected HC groups and the correlation between the use of a particular HC agent and the different histopathological subtypes, contingency analysis was performed (Pearson's chi-squared two-tailed test). For each analysis, the categorical outcome was defined as one of two possibilities (a specific subtype vs. another). A multivariate analysis (logistic regression) was conducted to evaluate the association of predictor variables with the

Fig. 1 Cohort selection scheme. A total of 656 patients were selected based upon adequate hormonal contraceptive information and adequate pathological data

likelihood of having benign pathology (no cancer) or other cancers (luminal A, luminal B, HER2, or triple negative). Calculations were set at a 95% confidence interval where $p < 0.05$ was considered statistically significant.

Results

Characteristics of Study Population Sixty-five percent of the patients in the study used hormonal contraceptive containing either of the following three agents LO, OTC, or ON, while 28% of the patients used an alternative single agent. Based on this, we narrowed our focus to the three specific agents (Fig. 2, Table 2). Additionally, seven cancer predisposing factors were selected for this study based on published literature: (1) ethnicity, (2) reproductive status (pre- or postmenopausal), (3) body mass index (BMI), (4) family history, (5) length of hormonal contraceptive utilization, (6) age at diagnosis, and (7)

time between last dose and diagnosis [3–11, 34, 47–52]. The details of the patient demographics, clinical and pathology data, and hormonal contraceptive use are listed in Table 3. Histopathological evaluation resulted in two thirds of the cases having benign pathology and one third of the cases being malignant. Mean duration of use of hormonal contraceptive agents was less than 1–5 years. The ethnicity was predominantly white; however, African Americans represented nearly 30% of the cohort analyzed. Over 74% of the cohort was premenopausal at diagnosis (Table 3).

Patients were first stratified by hormonal contraceptive (COC) agent into subpopulations of the total number of included patients ($n = 656$). The number of patients who had ever used a specific agent (e.g., OTC) was then compared against the populations that used any other formulations (e.g., ON, LO, and others). From a total of 4299 CBCP participants with both a completed patient questionnaire and a complete pathological diagnosis, 656 met the predetermined

Table 1 Parameters for determining histopathological subtypes

	ER	PR	HER2	Ki67
Benign	N/A	N/A	N/A	N/A
DCIS	±	±	N/A	N/A
Luminal A	+	±	–	–
Luminal B1	+	±	–	+
Luminal B2	+	±	+	+
Her2/neu	–	–	+	±
Triple negative	–	–	–	±

ER estrogen receptor, PR progesterone receptors, HER2 human epidermal growth factor receptor 2, Ki67 proliferation marker

Fig. 2 Distribution of hormonal contraceptive utilization among cohort

Table 2 Exogenous endocrine composition of the formulation groups: Lo Ovral, Ortho Tri-Cyclen, or Ortho Novum and number of patients assigned to these groups

Formulation	Exogenous endocrine component	<i>n</i>	Percent (%) of <i>n</i> = 656
Lo Ovral	Ethinyl estradiol + norgestrel	87	13.26
Ortho Tri-Cyclen	Ethinyl estradiol + norgestimate	87	13.26
Orthon Novum	Ethinyl estradiol + norethindrone	252	38.41
	Total	426	64.93

inclusion criteria for eligibility in this study (Fig. 1). Within the group of selected patients, over 50 different hormonal

Table 3 Demographic, pathologic, clinical, and hormonal contraceptive use characteristics for cohort (*n* = 656)

	Cases/category (%)
Age at diagnosis	
< 40 years	264 (40)
≥ 40 years	392 (60)
Ethnicity	
Caucasian	414 (63.1)
African American	179 (27.2)
Hispanic	32 (5)
Other	31 (4.7)
BMI	
< 18.5	8 (1.2)
18.5–24.9	301 (45.9)
25–29.9	210 (32)
≥ 30	129 (19.7)
Unknown	8 (1.2)
Menstruation status at diagnosis	
Premenopausal	490 (74.7)
Postmenopausal	166 (25.3)
Family history	
Positive	251 (38.3)
Negative	405 (61.7)
Number of HC agents	
1 agent	612 (94)
2 agents	38 (5.9)
3+ agents	6 (0.1)
Duration of HC use	
< 1–5 years	194 (29.6)
6–10 years	139 (21.2)
11–15 years	120 (18.3)
16–20 years	75 (11.4)
21–25 years	42 (6.4)
> 25 years	38 (5.8)
Unknown	48 (7.3)
Time between the last HC dose and diagnosis	
≤ 1 year	212 (32.3)
2–10 years	160 (24.4)
11–20 years	131 (20)
> 20 years	119 (18.1)
Unknown	34 (5.2)
Diagnosis	
Benign	411 (63)
Malignant	245 (37)
Pathology and subtyping	
Benign	411 (62.7)
DCIS	33 (5)
Luminal A	90 (13.7)
Luminal B	64 (9.7)
HER2/neu	11 (1.7)
Triple negative	47 (7.2)

contraceptive agents were identified. The top three formulations observed were LO (*n* = 87; 13.26%), OTC (*n* = 87; 13.26%), and ON (*n* = 252; 38.41%), constituting approximately 65% of the selected patients (Fig. 2). Importantly, each of these three top formulations—two of which were triphasic formulations (OTC and ON) and one was a monophasic formulation (LO)—was found to contain 30 (LO) or 35 µg (OTC, ON) ethinyl estradiol in addition to different progestins (LO = 0.3 mg norgestrel; OTC = 0.18/0.215/0.25 mg norgestimate; ON = 0.5/0.75/1 mg norethindrone; Table 2).

Patient Pathology Subtypes with Hormonal Contraceptive Use

The correlation between pathological subtypes and different hormonal contraceptive agents was determined and no significant difference was identified for the histopathological subtype in patients who utilized LO compared to the patients using any of the remaining agents (Table 4). However, OTC use showed a statistically significant correlation with benign ($p < 0.001$) and luminal A ($p = 0.0470$) lesions, and ON use showed a statistically significant correlation with benign lesions ($p = 0.0051$) and DCIS ($p = 0.0202$; Table 4). Trends for a negative association between DCIS in patients following OTC use ($p = 0.0754$) and a positive association of ON use with HER2 ($p = 0.0828$) and triple negative breast cancers ($p = 0.0643$; Table 4) were observed although not significant.

Patient Demographics and Hormonal Contraceptive Use

Patient variables were assessed between groups to determine if they impacted on the effects of LO, OTC, or ON on breast pathology development in our cohort. Age, ethnicity, BMI, menstruation status, duration of hormonal contraceptive use, time between last hormonal contraceptive dose, and diagnosis and initial diagnosis all showed statistically significant differences between the three hormonal contraceptive agents (Table 5). Most notably, patient age differed significantly between agents in that ever use of LO or ON strongly correlated with a diagnosis at the age of ≥ 40 years ($p = 0.0004$ and $p < 0.0001$, respectively), whereas use of OTC strongly correlated with a diagnosis at the age of < 40 years ($p < 0.0001$). When assessing patient ethnicity, Caucasians were less likely to have used OTC ($p = 0.0093$) and more likely to have used ON ($p = 0.0048$; Table 5). However, use of either formulation positively correlated with a premenopausal diagnosis ($p = 0.0015$ and $p < 0.0001$, respectively; Table 5) as well as the occurrence of benign condition ($p < 0.0001$ and $p =$

Table 4 Histopathological subtypes in relation to hormonal contraceptive agents

Pathological subtype	Non-LO <i>n</i> = 569		LO <i>n</i> = 87		<i>p</i> value
	No.	(% <i>n</i>)	No.	(% <i>n</i>)	
Benign	353	62	58	66.7	0.4059
DCIS	29	5.1	4	4.6	0.8428
Luminal A	81	14.2	9	10.3	0.3259
Luminal B	55	9.7	9	10.3	0.8425
HER2/neu	9	1.6	2	2.3	0.6276
Triple negative	42	7.4	5	5.8	0.5820
Pathological subtype	Non-OTC <i>n</i> = 569		OTC <i>n</i> = 87		<i>p</i> value
	No.	(% <i>n</i>)	No.	(% <i>n</i>)	
Benign	340	59.7	71	81.6	< 0.0001
DCIS	32	5.6	1	1.1	0.0754
Luminal A	84	14.8	6	6.9	0.0470
Luminal B	59	10.4	5	5.8	0.1760
HER2/neu	11	1.9	0	0.0	0.1909
Triple negative	43	7.6	4	4.6	0.3189
Pathological subtype	Non-ON <i>n</i> = 404		ON <i>n</i> = 252		<i>p</i> value
	No.	(% <i>n</i>)	No.	(% <i>n</i>)	
Benign	270	67	141	56	0.0051
DCIS	14	3.4	19	7.5	0.0202
Luminal A	50	12.4	40	15.9	0.2055
Luminal B	43	10.6	21	8.3	0.3321
HER2/neu	4	.9	7	2.8	0.0828
Triple negative	23	5.7	24	9.5	0.0643

Pearson chi-square two-tailed test to compare the correlation between pathological subtyping and three hormonal contraceptive agents; $p < 0.05$ is considered statistically significant

LO LO Ovral, OTC OrthoTri-Cyclen, ON Ortho Novum

0.0051, respectively; Table 5). Significant differences were observed for BMI across all three agents with each showing statistical difference within different BMI ranges: LO use was associated with a BMI of 18.5–24.9 ($p = 0.022$), OTC use was associated with a BMI of 25–29.9 ($p = 0.029$), and ON use was associated with a BMI of ≥ 30 ($p = 0.020$) (Table 5).

Duration of Hormonal Contraceptive Use and Breast Cancer Diagnosis Examination of duration of LO, OTC, or ON use and time between the last dose and breast pathology diagnosis revealed that these factors played significant roles in the diagnosis of breast pathologies. For example, LO use for 11–15 years was associated with breast pathology diagnosis and time between discontinuation of LO (i.e., < 1 or 11–20 years) was also associated with diagnosis of breast cancer pathology (Table 5). For OTC, less than 1 year and up to 10 years of

usage was associated with breast cancer diagnosis and 2 to > 20 years of time between last OTC use and diagnosis were also observed (Table 5). For ON, longer durations of use (i.e., 6–10 or 16–20 years) and less than 1 year or 11 to > 20 years of time between last ON dose and diagnosis were also observed (Table 5).

Hormonal Contraceptive Agent and Specific Pathological Subtype Given the fact that we identified differences in both pathological subtypes and patient variables between hormonal contraceptive agents (Table 4), we asked whether we could identify associations between patient factors across all hormonal contraceptive agents that contribute to a specific subtype of breast cancer. Table 6 outlines the results for all patients. When considering the ever use of any hormonal contraceptive agent, age at diagnosis was found to be statistically significant for benign ($p < 0.0001$), DCIS ($p = 0.0026$), luminal A ($p < 0.0001$), luminal B ($p = 0.0006$), and TN ($p = 0.0146$) pathologies. Specifically, women less than 40 years of age were more likely to have benign pathology compared to women 40 years or older. Women 40 or older were more likely to develop DCIS, luminal A, luminal B, and TN breast cancer. Ethnicity, specifically Caucasian and African Americans but not Hispanics, were significantly associated with luminal A and DCIS. Body mass index (BMI) was significantly associated with luminal A and HER2 breast cancer. A negative family history was associated with a benign diagnosis and a positive family history of breast cancer was associated with a diagnosis of luminal B breast cancer. Menstruation status at time of diagnosis was associated with luminal A, luminal B, and DCIS indicating that those diagnosed with benign, DCIS, luminal A, and luminal B pathologies were premenopausal. Time between the last dose and diagnosis was significant for luminal A, DCIS, and triple negative breast cancers; diagnosis of luminal A and DCIS occurred even after 20 years of discontinuation of these hormonal contraceptive agents and 2–20 years for triple negative breast cancer diagnosis. Duration of use was associated with luminal A and DCIS. Specifically, 1–5 or > 25 years of usage was associated with a diagnosis of luminal A breast cancer and 21–25 years of usage was associated with a diagnosis of DCIS (Table 6).

Patient Variables, Type of Hormonal Contraceptive, and Breast Cancer Pathologies Using the results from Table 5 as a baseline, contingency analyses were performed for LO, OTC, and ON to determine whether patient variables factored into the effect of LO, OTC, and ON on breast cancer pathologies as reported in Tables 7–9. For the subpopulation that had used LO (Table 7), the cohort that chose “other” (i.e., patients that did not identify as Caucasian, African American, or Hispanic), or patients with a BMI ≥ 30 or being postmenopausal at time of diagnosis or use of LO for > 25 years were more likely to develop DCIS. A HER2-positive subtype was more prominent

Table 5 Comparing hormonal contraceptive agents, patient variables and breast cancer diagnosis

	Non-LO <i>n</i> = 569 No. (%)	LO <i>n</i> = 87 No. (%)	<i>p</i> value	Non-OTC <i>n</i> = 569 No. (%)	OTC <i>n</i> = 87 No. (%)	<i>p</i> value	Non-ON <i>n</i> = 404 No. (%)	ON <i>n</i> = 252 No. (%)	<i>p</i> value
Age									
< 40 years	244 (43)	20 (33)	0.0004	200 (35.1)	64 (73.6)	< 0.0001	199 (49)	65 (26)	< 0.0001
≥ 40 years	325 (57)	67 (77)		369 (64.9)	23 (26.4)		205 (51)	187 (74)	
Ethnicity									
Caucasian	366 (64.3)	48 (55.2)	0.0995	370 (65)	44 (50.6)	0.0093	238 (59)	176 (69.8)	0.0048
African American	149 (26.2)	30 (34.5)	0.1057	152 (26.7)	27 (31)	0.3994	114 (28.2)	65 (25.8)	.4978
Hispanic	26 (4.6)	6 (6.9)	0.3480	27 (4.8)	5 (5.7)	0.6862	24 (5.9)	8 (3.2)	0.1097
Other	28 (4.9)	3 (3.4)	0.5466	20 (3.5)	11 (12.7)	0.0002	28 (6.9)	3 (1.2)	0.0008
BMI									
< 18.5	8 (1.4)	0	0.2658	6 (1)	2 (2.3)	0.3247	7 (1.7)	1 (0.4)	0.1295
18.5–24.9	271 (47.6)	30 (34.5)	0.0219	254 (44.6)	47 (54.1)	0.1019	196 (48.5)	105 (41.7)	0.0869
25–29.9	175 (30.8)	35 (40.2)	0.0777	191 (33.6)	19 (21.8)	0.0290	126 (31.2)	84 (33.3)	0.5668
≥ 30	109 (19.1)	20 (23)	0.4023	114 (20)	15 (17.2)	0.5415	68 (16.9)	61 (24.2)	0.0208
Unknown	6 (1.1)	2 (2.3)	0.3247	4 (0.8)	4 (4.6)	0.0021	7 (1.7)	1 (0.4)	0.1295
Menstruation status at diagnosis									
Premenopause	428 (75.2)	62 (71.3)	0.4293	413 (72.6)	77 (88.5)	0.0015	332 (82.2)	158 (62.7)	< 0.0001
Postmenopause	141 (24.8)	25 (28.7)		156 (27.4)	10 (11.5)		72 (17.8)	94 (37.3)	
Family history									
Positive	219 (38.5)	32 (36.8)	0.7603	225 (40)	26 (30)	0.0843	147 (36.4)	104 (41.3)	0.2107
Negative	350 (61.5)	55 (63.2)		344 (60)	61 (70)		257 (63.6)	148 (58.7)	
Duration of HC use									
< 1–5 years	173 (30.4)	21 (24.1)	0.2330	152 (26.7)	42 (48.3)	< 0.0001	120 (29.7)	74 (29.4)	0.9265
6–10 years	124 (21.8)	15 (17.2)	0.3333	128 (22.4)	11 (12.6)	0.0362	70 (17.3)	69 (27.4)	0.0022
11–15 years	97 (17)	23 (26.4)	0.0349	108 (19)	12 (13.8)	0.2438	75 (18.5)	45 (17.8)	0.8197
16–20 years	65 (11.4)	10 (11.5)	0.9846	70 (12.3)	5 (5.7)	0.0735	42 (10.4)	33 (13)	0.2906
21–25 years	35 (6.2)	7 (8.1)	0.5013	38 (6.7)	4 (4.6)	0.4603	33 (8.2)	9 (3.6)	0.0193
> 25 years	34 (6)	4 (4.6)	0.6084	35 (6.2)	3 (3.5)	0.3149	29 (7.2)	9 (3.6)	0.0544
Unknown	41 (7.2)	7 (8.1)	0.7792	38 (6.7)	10 (11.5)	0.1082	35 (8.7)	13 (5.2)	0.0936
Time between last HC dose and diagnosis									
≤ 1 year	194 (34)	18 (20.7)	0.0128	176 (31)	36 (41.4)	0.0523	180 (44.6)	32 (12.7)	< 0.0001
2–10 years	142 (25)	18 (20.7)	0.3881	129 (22.7)	31 (35.6)	0.0087	108 (26.7)	52 (20.6)	0.0769
11–20 years	101 (17.7)	30 (34.5)	0.0003	124 (21.8)	7 (8.1)	0.0028	56 (13.8)	75 (29.8)	< 0.0001
> 20 years	102 (18)	17 (19.5)	0.7160	114 (20)	5 (5.7)	0.0013	37 (9.2)	82 (32.5)	< 0.0001
Unknown	30 (5.3)	4 (4.6)	0.7915	26 (4.5)	8 (9.2)	0.0699	23 (5.7)	11 (4.4)	0.4555
Diagnosis									
Benign	353 (62)	58 (67)	0.4059	340 (60)	71 (81.6)	< 0.0001	270 (67)	141 (56)	0.0051
Malignant	216 (38)	29 (33)		229 (40)	16 (18.4)		134 (33)	111 (44)	

Table depicts differences in variables between patients who ever used a particular hormonal contraceptive and those that did not utilize that agent; $p < 0.05$ is considered statistically significant

LO LO Ovral, OTC OrthoTri-Cyclen, ON Ortho Novum

in patients using LO for 21–25 years and who were diagnosed 2–10 years following the last LO dose (Table 7).

For the subpopulation that had used OTC (Table 8) patients less than 40 years or with a negative family history of breast cancer was associated with a diagnosis of a benign neoplasm while patients with an age > 40 years at time of diagnosis, a menstruation status of being postmenopausal status at time of

diagnosis and over 25 years of OTC usage, were associated with luminal A breast cancer. A BMI ≥ 30, a positive family history of breast cancer as well as long-term (> 25 years) usage of OTC was associated with luminal B breast cancer. An age of diagnosis > 40 years, Caucasian and “other” ethnicity (i.e., non-African American or non-Hispanic), a high BMI (≥ 30), a positive family history of breast cancer and long-term

Table 6 Comparing LO, OTC and ON ever use, patient variables, breast pathology and molecular subtypes

	Benign	DCIS	HER2	Luminal A	Luminal B	Triple negative
Age at diagnosis						
< 40 years	< 0.0001	0.0026	0.1324	< 0.0001	0.0006	0.0146
≥ 40 years						
Ethnicity						
Caucasian	< 0.0001	0.0079	0.9709	0.0003	0.2087	0.2506
African American	0.0492	0.0448	0.4955	0.0149	0.4668	0.0786
Hispanic	0.0029	0.1819	0.4488	0.0741	0.9406	0.1071
Other	0.0816	0.6377	0.4563	0.2282	0.2093	0.5784
BMI						
< 18.5	0.0280	0.5125	0.7102	0.2565	0.3494	0.4292
18.5–24.9	0.0029	0.9595	0.0630	0.7679	0.0928	0.0215
25–29.9	0.4291	0.5493	0.0234	0.0976	0.3219	0.0533
≥ 30	< 0.0001	0.2592	0.9007	0.0033	0.1439	0.2936
Family history						
Positive	0.0113	0.6137	0.4495	0.1939	0.0044	0.7594
Negative						
Menstruation status at diagnosis						
Premenopausal	< 0.0001	0.0203	0.3949	< 0.0001	0.0394	0.4632
Postmenopausal						
Time between last dose and diagnosis						
≤ 1 year	0.0030	0.0748	0.0967	0.3215	0.3001	0.4786
2–10 years	0.3714	0.6627	0.1009	0.2964	0.1786	0.0227
11–20 years	0.6753	0.7921	0.5412	0.5755	0.3601	0.0123
> 20 years	< 0.0001	0.0201	0.9971	0.0006	0.2470	0.5625
Years of utilization						
1–5 years	< 0.0001	0.4911	0.1333	0.0168	0.1555	0.1041
6–10 years	0.3771	0.3947	0.8148	0.5649	0.8625	0.1266
11–15 years	0.5064	0.6320	0.1179	0.8918	0.6600	0.5829
16–20 years	0.3116	0.8985	0.4781	0.9177	0.4865	0.4390
21–25 years	0.1549	0.0046	0.1075	0.5661	0.9582	0.2140
> 25 years	< 0.0001	0.9461	0.4069	< 0.0001	0.0636	0.8573

Table depicts differences in pathological subtypes based on different patient variables as it pertains to the ever use of a hormonal contraceptive agent; $p < 0.05$ is considered statistically significant

LO LO Ovral, OTC OrthoTri-Cyclen, ON Ortho Novum

(> 25 years) use of OTC were associated with triple negative breast cancer (Table 8).

For those who used ON, ethnicity (i.e., Caucasian and African American) and 21–25 years of taking ON was associated with DCIS. For luminal A breast cancer, age at diagnosis of > 40 years, a BMI ≥ 30, and > 20 years between the last dose of ON and time of diagnosis were associated. For luminal B breast cancer, age at diagnosis ≥ 40 years, > 20 years between the last ON dose and diagnosis, and > 25 years of usage were associated. Premenopausal status and women ≥ 40 years were associated with a benign diagnosis (Table 9).

Multivariate analysis revealed that women above the age of 40 years are 3.9 times ($p < 0.001$) more likely to have breast cancer pathology than women below 40 years of age. Women with a BMI of ≥ 30 have 2.1 higher odds ($p = 0.017$) of having

cancer than women with BMI between 18.5 and 24.9. Women with negative family history of cancer were found to have 0.61 times lower odds ($p = 0.04$) of a breast cancer pathology than women with positive family history of breast cancer. Women who used contraceptives for longer than 25 years were 4.5 times ($p = 0.019$) more likely to have breast cancer pathology than women who used it for less than 1–5 years. Type of birth control, menopausal status, and ethnicity did not significantly predict breast cancer pathology (Table 10).

Discussion

Given the fact that one in eight women will develop breast cancer in their lifetime, it is imperative to further understand

Table 7 Comparing the ever use of Lo Ovral, patient variables, breast pathology, and molecular subtypes

	Benign	DCIS	HER2	Luminal A	Luminal B	TN
Age at diagnosis						
< 40 years	0.1495	0.2633	0.3584	0.3711	0.3711	0.8701
≥ 40 years						
Ethnicity						
Caucasian	0.6474	0.4143	0.1972	0.4943	0.9805	0.8231
African American	1.0000	0.1374	0.2993	0.5067	0.5067	0.7892
Hispanic	0.3694	0.5773	0.6970	0.5982	0.3885	0.5308
Other	1.0000	0.0156	0.7869	0.5493	0.5493	0.6634
BMI						
< 18.5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
18.5–24.9	0.0167	0.1374	0.6404	0.4138	0.4138	0.0947
25–29.9	0.5363	0.5248	0.7756	0.7854	0.7854	0.3531
≥ 30	0.0192	0.0114	0.4344	0.4360	0.4360	0.3517
Family history						
Positive	0.5294	0.1046	0.2751	0.2174	0.6146	0.0789
Negative						
Menstruation status at diagnosis						
Premenopausal	0.1802	0.0363	0.3636	0.7475	0.2714	0.6566
Postmenopausal						
Time between last dose and diagnosis						
≤ 1 year	0.5745	0.2956	0.4649	0.3227	0.4537	0.9687
2–10 years	0.0247	0.8275	0.0051	0.9046	0.0632	0.9687
11–20 years	0.5266	0.5390	0.2871	0.1048	0.8791	0.2412
> 20 years	0.4338	0.7268	0.4970	0.7540	0.5516	0.2742
Years of utilization						
1–5 years	0.1108	0.9671	0.4196	0.3348	0.8872	0.1939
6–10 years	0.5471	0.3500	0.5137	0.6071	0.6071	0.1652
11–15 years	0.2288	0.9468	0.4446	0.7620	0.1957	0.4787
16–20 years	0.8121	0.3859	0.6061	0.9696	0.9696	0.4065
21–25 years	0.5772	0.5447	0.0273	0.0987	0.3486	0.4957
> 25 years	0.0038	0.0461	0.7535	0.3245	0.3245	0.0903

Table depicts differences in pathological subtype based on different patient variables as it pertains to the ever use of Lo Ovral; $p < 0.05$ is considered statistically significant

patient factors associated with an increased risk of breast cancer development so that these risks can be minimized. Use of hormonal therapies has been associated with increased risk of breast cancer [13, 40] and the type of estrogen or progestin may play significant roles [12, 38, 40–46]. Inconsistent findings have been reported on the risk of breast cancer and hormonal contraceptive use confounded, perhaps, by the fact that many of the studies were conducted in postmenopausal women, which may have underestimated the risk of breast cancer in younger premenopausal women [13, 54–56].

This study takes a multilevel approach to evaluate both hormonal contraceptive agents and patient demographic factors to identify potential risks associated with breast pathology diagnosis—both benign and malignant. Our study determined that age at diagnosis, family history, menopausal status, duration of hormonal contraceptive use and time between the last

dose of hormonal contraceptive taken, and diagnosis played significant roles. With respect to duration of use and time between the last hormonal contraceptive use and breast pathology diagnosis, our findings are consistent with recent findings [13] demonstrating that risk of breast cancer increased from 1.09 with < 1 year of usage to 1.38 with > 10 years of use and that this risk remained high for those women taking hormonal contraceptives for ≥ 5 years. In our study, use of hormonal contraceptives in older women (≥ 40 years) may increase one's risk of DCIS, luminal A, luminal B, and triple negative breast cancers and being premenopausal may increase one's risk of DCIS, luminal A, and luminal B breast cancers. Patient variables may predict a specific subtyping between different hormonal contraceptive agents. For example, postmenopausal women taking LO had greater association with developing DCIS. Luminal A, luminal B, and triple

Table 8 Comparing the ever use of Ortho Tri-Cyclen, patient variables, breast pathology, and molecular subtypes

	Benign	DCIS	HER2	Luminal A	Luminal B	TN
Age at diagnosis						
< 40 years	0.0003	0.5465	1.0000	0.0011	0.4787	0.0241
≥ 40 years						
Ethnicity						
Caucasian	0.9594	0.3201	1.0000	0.0962	0.6262	0.0383
African American	0.5636	0.4999	1.0000	0.4305	0.6553	0.7894
Hispanic	0.9238	0.8039	1.0000	0.5308	0.1584	0.6131
Other	0.4159	0.7020	1.0000	0.3341	0.3809	0.0001
BMI						
< 18.5	0.4970	0.8774	1.0000	0.6970	0.7239	0.7535
18.5–24.9	0.0430	0.3535	1.0000	0.2919	0.1159	0.2331
25–29.9	0.3132	0.5950	1.0000	0.4800	0.9183	0.1628
≥ 30	0.0176	0.6462	1.0000	0.2795	0.0091	0.6741
Family history						
Positive	0.0517	0.5114	1.0000	0.0974	0.0004	0.0436
Negative						
Menstruation status at diagnosis						
Premenopausal	< 0.0001	0.7170	1.0000	< 0.0001	0.5391	0.0134
Postmenopausal						
Time between last dose and diagnosis						
≤ 1 year	0.0094	0.3981	1.0000	0.2027	0.0530	0.4959
2–10 years	0.8629	0.4543	1.0000	0.4463	0.2412	0.1276
11–20 years	0.4684	0.7661	1.0000	0.4211	0.3114	0.5447
> 20 years	0.0134	0.8039	1.0000	0.2336	0.5695	< 0.0001
Years of utilization						
1–5 years	0.0392	0.3312	1.0000	0.1083	0.1925	0.9437
6–10 years	0.9847	0.7020	1.0000	0.7586	0.6102	0.4360
11–15 years	0.1501	0.6874	1.0000	0.1503	0.6784	0.5057
16–20 years	0.9238	0.8039	1.0000	0.5308	0.1584	0.6131
21–25 years	0.3310	0.8252	1.0000	0.5773	0.6131	0.6531
> 25 years	0.0281	0.8492	1.0000	< 0.0001	0.6634	0.6988

Table depicts differences in pathological subtype based on different patient variables as it pertains to the ever use of Ortho Tri-Cyclen; $p < 0.05$ is considered statistically significant

negative breast cancers may be more associated with use of OTC along with older age, postmenopausal status, and positive family history. ON usage was more associated with luminal A and B breast cancers and this was dependent upon age and menopausal status. Given the fact that ON is also indicated for controlling vasomotor symptoms associated with postmenopausal women and prophylaxis for postmenopausal osteoporosis, it is not surprising that more postmenopausal women and women ≥ 40 years of age were diagnosed with luminal A and B conditions [57]. Considering that LO, OTC, and ON contain similar doses of ethinyl estradiol (30–35 µg) but different types of formulations (monophasic vs triphasic) and progestins (norgestimate, norethindrone, norgestrel) raises the possibility that the formulation type and progestin component may have some role in the observed pathological diagnosis in other studies [12, 38, 41–46].

With respect to estrogen, low-dose estrogen (i.e., 20 µg) oral contraceptives were not associated with an increase in breast cancer risk whereas moderate (30–35 µg) and high dose (50 µg) were associated with a 1.6× or 2.7× elevations in breast cancer risk, respectively [34]. All of the oral contraceptive agents examined in our study would be classified as moderate dose because they contained between 30 and 35 µg ethinyl estradiol.

Our findings that LO use was associated with the least amount of diagnoses draw attention to the type of formulation since it was the only monophasic formulation compared to OTC and ON, which are triphasic formulations. Triphasic formulations containing levonorgestrel showed a relative risk (RR) of 3.3 in the Nurses' Health Study II [35] and a RR of 1.8 in the study by Beaber et al., [34]. Earlier studies have shown that norgestimate (OR, 1.2; 95% CI, 0.6–2.2) was not

Table 9 Comparing the ever use of Ortho Novum, patient variables, breast pathology, and molecular subtypes

	Benign	DCIS	HER2	Luminal A	Luminal B	TN
Age at diagnosis						
< 40 years	< 0.0001	0.1137	0.1137	0.0039	0.0214	0.1176
≥ 40 years						
Ethnicity						
Caucasian	0.0387	0.0139	0.1146	0.0572	0.8685	0.7216
African American	0.2923	0.0334	0.0545	0.1912	0.8282	0.3748
Hispanic	0.0678	0.4117	0.6271	0.2118	0.6648	0.3510
Other	0.1221	0.6188	0.7684	0.4491	0.5993	0.5719
BMI						
< 18.5	0.3740	0.7748	0.8655	0.6634	0.7626	0.7451
18.5–24.9	0.2740	0.3134	0.1362	0.8157	0.7288	0.1916
25–29.9	0.5903	0.8660	0.1753	0.1130	0.1469	0.3626
≥ 30	0.3537	0.3730	0.7845	0.0324	0.2676	0.5509
Family history						
Positive	0.4109	0.2955	0.4889	0.3829	0.8774	0.6933
Negative						
Menstruation status at diagnosis						
Premenopausal	0.0010	0.0536	0.2709	0.0302	0.3072	0.6726
Postmenopausal						
Time between the last dose and diagnosis						
≤ 1 year	0.1186	0.7674	0.3061	0.2817	0.8195	0.4995
2–10 years	0.2208	0.9627	0.5987	0.3370	0.8511	0.3005
11–20 years	0.2627	0.0565	0.4422	0.4727	0.1052	0.0702
> 20 years	0.0005	0.0519	0.8202	0.0277	0.0427	0.7108
Years of utilization						
1–5 years	0.6567	0.8256	0.3743	0.7777	0.3589	0.3346
6–10 years	0.8628	0.5200	0.4307	0.9853	0.8983	0.2425
11–15 years	0.5462	0.3856	0.0799	0.4032	0.0256	0.8728
16–20 years	0.8403	0.2926	0.2183	0.2528	0.8659	0.2374
21–25 years	0.0379	< 0.0001	0.6056	0.6906	0.7588	0.3216
> 25 years	0.1639	0.3830	0.6056	0.1444	0.0057	0.3216

Table depicts differences in pathological subtype based on different patient variables as it pertains to the ever use of Ortho Novum; $p < 0.05$ is considered statistically significant

associated with an increased risk of breast cancer but levonorgestrel oral contraceptives were (OR 1.5; 95% CI 1.0–2.3) [34]. Monophasic moderate-dose estrogen + 0.5 mg norethindrone demonstrated no increase in risk (0.8 or decrease in risk) whereas moderate dose estrogen + 1.5 mg norethindrone was associated with a 2.1 increase in breast cancer risk suggesting that risk increased with increasing norethindrone dose (1.0 vs 0.5 mg) [34]. Triphasic oral contraceptives with an average dose of norethindrone (0.75 mg) had the greatest risk of 3.1×; however, this formulation was uniquely different. In the Nurses' Health Study II, they demonstrated that breast cancer risk increased in women less than 55 years who were current oral contraceptive users and one triphasic formulation containing levonorgestrel accounted for much of the risk (3.1×) [35]. In the Beaver study [34], moderate estrogen dose and 0.09 mg levonorgestrel demonstrated a 1.8× increase in risk.

Besides the formulation, the type of progestin contained within the oral contraceptive may play a role. Medroxy progesterone acetate (MPA) has been shown to increase breast cancer risk in the Million Women Study and the Women's Health Initiative [41] and so progestins with specific characteristics (e.g., pharmacological, metabolic, structural) like MPA may increase breast cancer risk [58]. Using rodent models, MPA has the characteristics of possessing high progestogenic properties (++), medium glucocorticoid properties (+), and low to no androgenic properties (±) with no anti-androgenic (–) or anti-mineralocorticoid properties (–) [58]. Norethindrone (the progestin contained within ON) possesses similar characteristics as MPA with respect to progestogenic (++), anti-androgenic (–), and anti-mineralocorticoid (–) properties but differs from MPA by possessing androgenic (+) but no glucocorticoid (–) properties. For levonorgestrel

Table 10 Multivariate analysis of the three OC groups and patient variables ($n = 426$)

	$n = 426$ No. (% n)	Odds ratio (95% CI)	p value
Oral contraceptives			
Lo Ovral	87 (20)	Reference	
Ortho Tri-Cyclen	252 (60)	0.93 (0.48, 1.4)	0.862
Ortho Novum	87 (20)	1.6 (1.3, 1.9)	0.097
Age			
< 40 years	149 (35)	Reference	
≥ 40 years	277 (65)	3.9 (3.6–4.2)	< 0.0001
Menopausal status			
Premenopausal	297 (70)	Reference	
Postmenopausal	129 (30)	1.6 (1.3, 1.8)	0.072
Ethnicity			
Caucasian	268 (63)	Reference	
African American	122 (29)	0.85 (0.58, 1.1)	0.548
Hispanic	19 (5)	0.30 (– 0.41, 1.0)	0.088
Other	17 (4)	0.94 (0.20, 1.7)	0.939
BMI			
18.5–24.9	182 (43)	Reference	
25–29.9	138 (32)	1.7 (1.4, 2.0)	0.056
≥ 30	96 (23)	2.1 (1.8, 2.4)	0.017
Family history			
Positive	162 (38)	Reference	
Negative	264 (62)	0.61 (0.41, 0.85)	0.041
Years of utilization			
< 1–5 years	137 (32)	Reference	
6–10 years	95 (23)	1.1 (0.78, 1.4)	0.762
11–15 years	80 (19)	1.4 (1.0, 1.7)	0.350
16–20 years	48 (11)	1.3 (0.89, 1.7)	0.531
21–25 years	20 (5)	1.7 (1.2, 2.2)	0.318
> 25 years	16 (4)	4.5 (3.9, 5.2)	0.019

$p < 0.05$ is considered statistically significant

(the progestin contained within LO), it is similar to MPA with respect to progestogenic properties (++) and anti-androgenic properties (–) but differs from MPA with respect to displaying androgenic (+) properties, no glucocorticoid (–) properties, and low to none ($\pm$) anti-mineralocorticoid properties [58].

OTC and LO contain progestins classified as gonanes and ON contains a progestin classified as an estrane [59]. Estrane progestin contraceptives have been shown to increase breast cancer risk by 60% while gonane progestin oral contraceptives demonstrated a 40% increase in breast cancer risk [34]. Norgestimate did not appear to be associated with an increase in breast cancer risk (20% increase) but levonorgestrel oral contraceptives were associated with a 50% increase in risk although neither odds ratio was statistically different than using other progestin types [34].

Although all progestins are structurally related to testosterone, the progestin contained within OTC (norgestimate) is more progestogenic compared to ON and LO, which contain progestins that are more androgenic in nature [58]. The rank order of potency (from most potent to least potent) for the progestins based on ovulation inhibition potency is levonorgestrel (LO) > norgestimate (OTC) > norethindrone (ON) [59]. With respect to potency to affect the endometrium in postmenopausal women, levonorgestrel (LO) was the most potent followed by norethindrone (ON) while MPA fell in between [59], which is consistent with their binding profile to PRs (from most potent to least potent: levonorgestrel > MPA > norethindrone > progesterone) [58]. Although it has been suggested that the androgenic component may underlie the cancer-inducing effects of progestins, studies demonstrate that levonorgestrel and norethindrone, like MPA, increases factors such as cancer diagnosis, processes of angiogenesis, VEGF expression via PR/PREs, vascular inflammation by inducing adhesion molecule-1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1) via PRs [58].

The pharmacokinetic parameters of OTC, ON, and LO differ, which may contribute to the pathologies observed in response to the oral contraceptives. Like MPA, both norethindrone and norgestrel, the progestins contained within ON and LO, respectively, undergo A-ring reduction forming both sulfated and glucuronidated versions that can be converted back to their active forms by sulfatases or metabolized to ethinyl estradiol [59]. This may impact on their half-lives in the body which are $t_{1/2} = 5–13$ h for ON and $t_{1/2} = 10–13$ h for LO [59]. OTC is metabolized to levonorgestrel and levonorgestrel-3-oxide [59].

Current literature provides mixed data regarding oral contraceptive use and type of breast cancer diagnosed (ER+ vs ER–). Our study, together with that of Beaber et al., 2014 [34] and Huang et al., 2000 [60] showed strong association of ER+ breast cancer with oral contraceptive use. However, Althuis et al. 2003 [25] and Dolle et al., 2009 [48] did not find such an association with ER+ breast cancer but did find an association with ER– breast cancer. Rosenberg et al., 2010 [33] and Sweeney et al., 2007 [61] also demonstrated a greater risk for ER– breast cancer. Also, Ma et al., 2010 [62] found no association with luminal A and triple negative breast cancer contrary to our observations with both pre- and postmenopausal women.

In our study, age, family history of breast cancer, BMI (≥ 30) and duration of oral contraceptive use were observed to increase the risk of breast cancer. With respect to BMI, women taking LO had a lower BMI compared to women taking OTC (BMI range 25–29.9) and ON (BMI > 30) and women taking LO had the least amount of breast cancer pathologies. Duration of oral contraceptive use (> 25 years) was also associated with breast cancer pathology and this is in line with earlier findings that found risk of overall breast cancer and ER+ cancer increased with increased number of pills dispensed over the prior year [34].

While utilizing patient questionnaires and pathological diagnoses from patients enrolled in the CBCP provided an adequate sample size for this retrospective study, there were however limitations. First, the accuracy of information documented in the core questionnaire is dependent upon patient self-report and appropriate documentation by the interviewing health care provider. Additionally, the large exclusion group was precipitated by inconsistencies in spelling and documentation of the formulation's name or lack thereof. These inconsistencies may have implications on the information we have regarding the true number of agents the patient had ever used along with the duration of each agent. Pertinent information, such as dose and compliance, was not among the data captured in the questionnaire and therefore could not be considered a predictor in this study. The required exclusions due to inadequate information reduced our total sample size.

Overall, the findings from this study provide valuable information on the association between patient factors (such as family history of breast cancer, BMI, age, and duration of use) and effects of the combination of oral contraceptive on breast pathology—benign and malignant. These factors along with duration of use should be considered when choosing a combined hormonal contraceptive for use in women to reduce their risk of developing breast cancer.

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Compliance with Ethical Standards

Appropriate IRB approval was obtained for the Clinical Breast Care Project (CBCP) from the study sites at the Walter Reed National Military Medical Center and Windber Research Institute/Windber Medical Center. IRB approval was also obtained from Duquesne University School of Pharmacy and Division of Pharmaceutical, Administrative and Social Sciences.

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