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Breast cancer and hormonal intake among Egyptian females

Tumore al seno e assunzione di ormoni in donne egiziane

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Summary

Background. **Carcinoma of the breast is the most prevalent cancer among Egyptian women and represents 29% of the National Cancer Institute cases. Cancer of the breast could be enhanced by many factors; one of them is estrogen, whether endogenous or exogenous. Epidemiological evidence indicates that prolonged lifetime exposure to estrogen is associated with elevated breast cancer risk in women.** ***Aim.*** **To study the association between breast cancer and history of intake of reproductive hormones (estrogen, progesterone and hormonal replacement therapy) among Egyptian females and identify other risk factors for breast cancer.** ***Patients and Methods.*** **A case-control study was carried out in the National Cancer Institute and Ain Shams University hospitals in Cairo. A total number of 351 patients were included, 198 breast cancer cases and 153 controls. All cases and controls underwent interviewing questionnaire inquiring about risk factors for breast cancer including: socio-demographic, reproductive history and environmental exposures. Anthropometric measurements, pathological staging and typing of tumour were determined. Immuno-histochemistry study was carried out to detect the profile of estrogen and**

Riassunto

Contesto. **Il carcinoma della mammella è il tumore più diffuso tra le donne egiziane e costituisce il 29% dei casi del National Cancer Institute. Il cancro al seno potrebbe essere favorito da molti fattori, uno dei quali è rappresentato dagli estrogeni, sia endogeni che esogeni. Le evidenze epidemiologiche indicano che l'esposizione prolungata agli estrogeni è associata ad elevato rischio di cancro al seno nelle donne.** ***Finalità.*** **Studiare l'associazione tra il carcinoma della mammella e la storia di assunzione di ormoni riproduttivi (estrogeni, progesterone e terapia ormonale sostitutiva) tra le donne egiziane.** ***Pazienti e Metodi.*** **È stato condotto nel National Cancer Institute e negli ospedali della Ain Shams University del Cairo uno studio caso-controllo che includeva 351 pazienti in totale, di cui 198 casi di cancro al seno e 153 controlli. Tutti i casi e i controlli sono stati sottoposti ad un questionario-intervista per la ricerca di fattori di rischio per il cancro al seno, tra cui: storia socio-demografica, riproduttiva e esposizioni ambientali. Sono state determinate misure antropometriche, tipizzazione e stadiazione patologica del tumore. Sono stati effettuati saggi immunoistochimici per individuare il profilo dei recettori degli estrogeni e del progesterone e sono**

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progesterone receptors and blood samples were collected in order to determine the levels of estrogen and progesterone. *Results.* The breast cancer cases showed a statistical significant difference in comparison with the controls regarding the current or past exposure to passive smoking at home (p<0.001), higher menstrual irregularities at the age of menarche, more exposure to insecticides (p<0.05) and domestic chemicals (p<0.001), living near a factory (p<0.05), and history of exposure to breast trauma (p<0.05) and in particular brest feeding related to one of the breasts (p<0.05). No significant difference was found as regards the marital status, parity, age at menarche, age at menopause, age at first birth, lactation, body mass index grades and waist hip ratio. Higher ever use of reproductive hormones was observed among cases compared to controls (60.6%vs 52.9%) but the difference was statistically no significant (OR 1.4 CI: 0.9-2.1). The majority of breast tumours were of the ductal subtype and the profile of hormone receptors is positive for estrogen receptors and/or progesterone receptors in less than half of cases. Estrogen and progesterone receptors percentage increases with the increasing age groups. Progesterone receptor was expressed mostly among the group having no lymph nodes and no metastases involved, and its expression increases as the tumour size. No significant difference was found regarding the type of tumour and the TNM classification of breast cancer between cases with or without positive history of intake of reproductive hormones. *Conclusions.* Among all the epidemiological variables studied passive smoking (current and past), exposure to insecticides, domestic chemicals and breast trauma were the most significant factors associated with breast cancer. No relationship was observed between the expression of estrogen and progesterone receptors and history of intake of reproductive hormones in breast cancer cases. Eur. J. Oncol., 14 (1), 37-51, 2009

Key words: **Breast cancer, reproductive hormones, estrogen and progesterone receptors**

stati raccolti campioni di sangue per quantificare i livelli dei 2 ormoni. *Risultati.* I casi di cancro al seno hanno mostrato una differenza statisticamente significativa rispetto ai controlli per quanto riguarda l'attuale o la passata esposizione al fumo passivo in casa (p<0,001), una maggiore irregolarità mestruale all'età del menarca, l'esposizione agli insetticidi (p<0,05) e ai prodotti chimici domestici (p<0,001), vivere in prossimità di una fabbrica (p<0,05), una storia di traumi al seno (p<0,05) e in particolare l'allattamento al seno relativamente a uno dei due seni (P<0,05). Non è stata rilevata una differenza statisticamente significativa per quanto riguarda lo stato civile, il numero di figli partoriti, l'età al menarca, l'età della menopausa, l'età al primo parto e l'allattamento, l'indice di massa corporea e il rapporto vita-fianchi. Nessuna differenza statisticamente significativa (OR 1,4 CI: 0,9-2,1) è stata osservata tra i casi ed i controlli (60,6% vs 52,9%) per quanto riguarda la storia di assunzione di ormoni riproduttivi. La maggior parte dei tumori della mammella appartengono al sottotipo duttale e il profilo dei recettori ormonali è positivo per i recettori degli estrogeni e/o del progesterone in meno della metà dei casi. La percentuale dei recettori degli estrogeni e del progesterone aumenta con l'aumentare dell'età nei gruppi. Il recettore per il progesterone è stato espresso soprattutto tra i gruppi che non hanno linfonodi e metastasi coinvolti, e la sua espressione aumenta con l'aumentare delle dimensioni del tumore. Non è stata trovata una differenza significativa per quanto riguarda il tipo di tumore e la classificazione TNM del carcinoma della mammella tra i casi con o senza storia di assunzione di ormoni riproduttivi. *Conclusioni.* Tra tutte le variabili epidemiologiche studiate, il fumo passivo (attuale e passato), l'esposizione a insetticidi, a prodotti chimici domestici e i traumi al seno sono stati i fattori più significativi associati al carcinoma della mammella. Nei casi di cancro al seno non è stata osservata alcuna relazione tra l'espressione dei recettori degli estrogeni e del progesterone e la storia di assunzione di ormoni riproduttivi. Eur. J. Oncol., 14 (1), 37-51, 2009

Parole chiave: **tumore al seno, ormoni riproduttivi, recettori degli estrogeni e del progesterone**

Introduction

Cancer is now considered a world health problem. Cancers in women represent about 25% of all cancer cases discovered in the period between 1999-2001 in Egypt (1). Carcinoma of the breast is the most prevalent cancer among Egyptian women and constitutes the 29% of the National Cancer Institute cases and the 33% by the Alexandria Cancer Registry (2, 3).

Several studies in Egypt have reported high rates of breast cancer among women (4). Specific attributes of family and personal history are the most significant indicators of an increased risk of cancer in the individual patient (5).

Cancer of the breast could be enhanced by many factors; one of them is estrogen, whether endogenous or exogenous (6). Many studies have shown that oral contraceptive (OC) use is associated with an increase in a young woman's risk of breast cancer, although some studies suggest that the risk may be limited to recent use (6-9). A recent study in the US reported a 1.5 excess risk from OC use (10). Some data suggest that hormone replacement therapy (HRT) with estrogen is associated with increased risk of developing breast cancer, which is proportionate to the duration of use (7). However, the degree of association between breast cancer and postmenopausal hormonal therapy (HT) remains controversial (11). Ross and his colleagues (7) provide strong evidence in a case control study that the addition of a progestin to HRT enhances markedly the risk of breast cancer (OR = 1.24) relative to estrogen use alone (OR = 1.1). These findings have important implications for the risk-benefit equation for HRT in women using combined hormonal replacement therapy (CHRT). Current users of hormone use were more likely than never users to die from breast cancer (RR = 1.22) (9).

The relationship between clinically apparent breast cancer and postmenopausal HT has been investigated in the last few years. Most women using HT for short-term treatment of vasomotor symptoms will not face measurable increases in their risk for breast cancer, even if long-term current users are at increased risk (12). That risk returns to baseline a few years after discontinuation of hormones (12). There are still unanswered questions in this issue: if different types and routes of estrogen and progesterone, the duration and cessation of HT use have different impacts on breast cancer (11). Endogenous estrogen and progesterone could be assayed in blood while exogenous hormonal intake can only be assessed by thorough history intake. In 2002 the Women's Health Initiative concluded definitely that combined estrogen and progestin therapy increases the risk of invasive breast cancer. A recent re-analysis of over 90% of breast cancer studies throughout the world showed an increased risk in breast cancer for women who used postmenopausal hormones for 5 years or longer.

The aim of this work is to study the association between breast cancer and history of intake of reproductive hormones (estrogens and progesterone) among Egyptian females controlling for other known risk factors.

Subjects and Methods

Study design and setting

A case-control study was carried out at the National Cancer Institute (NCI) and Ain Shams University Hospitals during the period from October 2005 to October 2006.

Patients

Breast cancer cases (198) were recruited from the 6 Surgical departments of the National Cancer Institute in Cairo.

All newly diagnosed cases fulfilling the inclusion criteria were surveyed on a daily basis during the estimated period of the execution phase, and included in the study after obtaining an informed consent. A breast cancer patient was identified if postoperative pathological examination revealed a malignant breast mass, any case proven to be benign was excluded from the study.

Inclusion criteria for selection of cases were represented by age <60 years, no family history of breast or ovarian cancer, no previous history of breast cancer, no occupational exposure to ionizing radiation. These criteria were considered to control all

factors that would confound the role of exogenous hormones.

Controls were defined as apparently healthy females (153), with no previous benign breast disease. They were selected from the patients at the Department of Ophthalmology, Ain Shams University hospitals. They were group matched for age with breast cancer cases. Inclusion criteria for selection of controls were the same as cases except for the presence of any breast lump.

Sample size

Sample size was calculated taking into consideration the prevalence of the least prevalent risk factors in the general population, particularly the intake of hormonal contraceptives. Such figure was obtained after a pilot study on females with and without breast cancer to estimate the extent of their use for exogenous estrogens (which was found to be 7%). In addition, experts from Gynaecology and Obstetric Department in Ain Shams University Hospitals, were asked to rate the prescription of HRT in the form of estrogen for menopausal patients in their private and public clinics. They reported a rate ranging between 7 and 15%. The power of the test was set at 80% and the confidence interval at 95%. Alpha error was set at 0.05. Assuming an association between female breast cancer and history of intake of reproductive hormones with an odds ratio of 2, the calculated sample size was 150, for a ratio of 1:1 case/control (a total of 300 cases and control). All calculations were performed using the 6th version of Epi-Info statistical package.

Tools

All cases and controls underwent the following steps:

I-Interview questionnaire

It was designed including questions about socioeconomic data, special habits such as active and passive smoking, reproductive history, history of intake of reproductive hormones such as contraceptive hormones, HRT, and family history of the diseases.

II-Anthropometric measures

Some anthropometric measurements were determined for both cases and controls. Measurements were carried out with privacy at the hospital ward by a female doctor.

Body weight was measured using a spring balance scale for the nearest 0.5 kg, with light clothing and no shoes and the balance was regularly titrated by known weights. Standing height was measured to the nearest 0.5 cm while the subject is bare footed. Then body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of the height in meters (kg/m^2). According to the American Society of Nutrition, normal weight is a BMI between 18.5 and 25, overweight is a BMI 25 and 30 and obesity is a BMI ≥30 (13). Waist circumference was measured just over the iliac crest of the pelvis at the level of umbilicus (12). Hip circumference was measured at the maximal protrusion of the buttocks (14). Waist and hip circumferences were taken to the nearest centimeter with a flexible measuring tape and then waist/hip ratio (WHR) was obtained by dividing the waist circumference by the hip circumference. Women with a WHR 0.80-0.84 were classified as centrally overweight, while women with a WHR ≥0.85 were classified as centrally obese.

III-Laboratory Investigations

- A sample of the breast cancer tissue was drawn by the surgeon during the operation to investigate the pathology staging and typing of the tumour and the pathological TNM system, the most widely used means for classifying the extent of cancer spread which is the basis for prognostic assessment.
- Immunohistochemistry was performed to detect the estrogen and progesterone receptors.
- Blood sample was collected from both cases and controls for measuring estrogen and progesterone serum levels by the electrochemiluminescence immunoassay on the Roche Elecsys 2010 immunoassay analyzer (among premenopausal patients: 63 cases, 67 controls; among postmenopausal patients: 83 cases, 66 controls).

Statistical analysis

The data were revised for completeness and consistency: data entry, data checking and data analysis. The collected data were coded, tabulated and then statistical analysis was performed using computer programmes: Access Computer Package, SPSS Package version 12 and Microsoft Word Document 2003. Suitable statistical tests were applied. Also a descriptive analysis was carried out for each quantitative variable and the difference between case and control groups was assessed using t test for quantitative data, and Chi square tests for qualitative data.

Ethical considerations

Written informed consent was obtained from all the study patients after explanation of the study objectives. Special room was processed for anthropometric measurements to assure privacy of all patients. Confidentiality of all data was kept for all patients by the principal investigator.

Results

A total number of 351 cases and controls (198 cases, 153 controls) were recruited. More cases were included after revising the collected data, to replace data that were missed in the completed questionnaires.

Socio-demographic characteristics

The mean age of cases was 46.3±8.3 years and 45.9±8.0 years for the control group (Table 1). No significant difference in the age between cases and controls ($p>0.05$). The distribution of breast cancer cases and controls was carried out in accordance with the level of education ($p>0.05$).

Regarding the residence, table 1 also shows no significant difference between cases and controls as regards rural/urban, living near high voltage power lines or a cell phone station, but more breast cancer cases were living near a factory, such as factory of cement, aluminum, iron, electric home appliances, and textiles than controls (OR=1.9, 95% CI: 1.0 to 3.4, $p<0.05$).

No statistically significant difference between cases and controls was observed as regards the marital status, number of children, nor employment ($p>0.05$); some cases had late age at first marriage but not statistically significant.

Table 2 shows the distribution of breast cancer cases and controls according to cigarette smoking. It shows that the breast cancer cases had a statistically significant difference in current or past history of exposure to passive smoking at home (husband) (OR=1.9 95% CI: 1.2 to 3.0, $p<0.001$ and, OR=3.2 95% CI: 2.0 to 5.1, $p<0.001$ respectively) but the duration of exposure was not significant.

Regarding the history of exposure to radiation and surgery, it shows no significant difference between cases and controls except for the cases that were more exposed to breast trauma (OR=3.0 95% CI: 0.9 to 9.3, $p<0.05$); no significant difference is also shown between both groups regarding history and age of mammography and number of times, history of exposure to x-rays, history of radiotherapy, surgeries in breast as needle biopsy, partial breast excision, history of benign or malignant breast tumours, hysterectomy and age at hysterectomy.

Table 3 shows the history of exposure to environmental, chemical, and insecticides. Breast cancer cases were more exposed to insecticides and chemicals (chlorine, phenol benzene) than controls (OR=2.0 95% CI: 1.2 to 3.5, $p<0.01$ and OR=3.2, 95% CI: 2.2 to 5.2, $p<0.001$ respectively).

Tumour was discovered by the patient herself in 97% (n=191) while 86.4% (n=172) reported no past history of medical problems in the affected breast.

Table 4 shows the distribution of breast cancer cases and controls according to reproductive history. It shows no significant difference between cases and controls as regards the age of menarche, irregularity of menses, duration of menstrual cycle, menopausal status and age at menopause; no difference as regards the age at first or last pregnancy, mean duration of breast feeding. Breast cancer cases significantly differed in having menstrual irregularity early at the age of menarche (OR=0.52 95% CI: 0.39 to 0.68, $p<0.05$.

Table 5 shows the distribution of breast cancer cases and controls according to the intake of repro-

Table 1 - Distribution of breast cancer cases and controls according to socio-demographic data

Variables	Controls n=153	Cases n=198	p value	OR (95% CI)
Mean age (years) Mean ± SD	45.9 ± 8.0	46.3 ± 8.3	> 0.05	
Level of Education: N (%)				
• Illiterate to Prep. school	123 (80.4)	162 (81.8)	> 0.05	
• Secondary to University	30 (19.6)	36 (18.2)		
Residence: N (%)				
• Rural	48 (31.4)	70 (35.4)	> 0.05	
• Urban	105 (68.6)	128 (64.6)		
Living near a factory*: N (%)	18 (11.8)	41 (20.7)	0.032*	1.9 (1.0 to 3.4)
Living near high voltage electric cables: N (%)	27 (17.6)	23 (11.6)	> 0.05	
Living near cell phone station: N (%)	18 (11.8)	21 (10.6)	> 0.05	
Marital Status: N (%)				
• Non married	3 (2.0)	2 (1.0)		
• Married	113 (73.9)	149 (75.3)	> 0.05	
• Widow	32 (20.9)	34 (17.2)		
• Divorced & separated	5 (3.3)	13 (6.6)		
Mean duration of marriage (years) Mean±SD	25.0 ± 10.0	23.7 ± 10.6	> 0.05	
Parity (nulliparous): N (%)	n=150 3 (2.0)	n=196 8 (4.1)	> 0.05	
Mean age at first marriage (years) Mean ± SD	18.6 ± 4.6	20.6 ± 5.4	> 0.05	
No of children Mean ± SD	n=145 4.1 ± 1.8	n=187 3.8 ± 1.6	0.07	
Employment: N (%)				
• Working for cash	38 (24.8)	46 (23.2)	> 0.05	
• Housewife	115 (75.2)	152 (76.8)		

* Type of factory as: cement, aluminium, iron, electric home appliances, and textiles

ductive hormones. The duration of hormonal treatment for menstrual irregularties ranged from 3 to 120 months. No significant difference was observed among cases and controls as regards the intake of hormonal contraception, mean duration of intake, intake of HRT. Higher intake of exogenous reproductive hormones was observed among cases compared to controls (60.6% vs 52.9%) but the difference was statistically no significant (OR=1.4 95% CI: 0.09-2.1). The breast cancer cases showed no significant difference of median duration of hormonal treatment for menstrual irregularity ($p<0.05$).

Table 6 shows that there is no statistically significant difference between cases and controls as regards the mean serum level of estrogen in both pre-

Table 2 - Distribution of breast cancer cases and controls according to cigarette smoking and history of breast trauma

Variables	Controls n=153	Cases n=198	p value	OR (95% CI)
Cigarette smoking smoking status: N (%)				
• Non smokers	150 (98.0)	191 (96.5)		
• Current smokers	0	3 (1.5)	>0.05	
• Ex-smokers	3 (2.0)	4 (2.0)		
Current exposure to passive smoking at home: N (%)	71 (46.4)	125 (63.1)	<0.001*	1.9 (1.2 to 3.0)
Index Smoker: N (%)	n=71	n=117		
• Husband	47 (66.2)	92 (78.6)	0.06 NS	
• Son	31 (44.7)	47 (40.2)	>0.05	1.8 (0.9 to 3.6)
• Other family members	2 (2.8)	12 (10.3)	0.016*	
Mean age at start of current exposure (years) Mean±SD	n=73 26.4 ± 13.8	n=113 21.9 ± 11.6	0.02*	
Past history of exposure to passive smoking at home: N (%)	n=149 47 (31.5)	n=193 116 (60.1)	<0.001*	3.2 (2.0 to 5.1)
Mean duration of exposure to past passive smoking (years) Mean±SD	n=47 19.3 ± 8.2	n=105 21.1 ± 10.0	>0.05	
History of breast trauma: N (%)	4 (2.6)	15 (7.6)	0.042*	3.0 (0.9 to 9.3)

* Statistically significant

Table 3 - Distribution of breast cancer cases and controls according to exposure to environmental chemicals and insecticides

Variables	Controls n=153	Cases n=198	p value	OR (95% CI)
Exposure to chemicals[§]: N (%)	44 (28.8)	113 (57.1)	<0.001*	3.2 (2.2 to 5.2)
Exposure to insecticides: N (%)	24 (15.7)	55 (27.8)	0.007*	2.0 (1.2 to 3.5)
Home exposure to chemicals: N (%)	n=44 41 (93.2)	n=113 109 (96.5)	>0.05	
Duration of exposure (years) Mean±SD	n=44 10.0 ± 6.2	n=113 18.5 ± 11.4	<0.001*	

[§]Chemicals as chlorine, phenol, benzene (All exposures to insecticides occurred at home)
* Statistically significant

menopausal in the different phases of the menstrual cycle, and postmenopausal females. On the contrary, the mean serum level of progesterone was higher among cases than controls and the difference was borderline in follicular phase in premenopausal and postmenopausal females (p=0.05).

Table 7 shows the distribution of breast cancer cases and controls according to anthropometric measurements in pre and postmenopausal women. No statistically significant difference between cases and controls as regards the BMI grades, WHR among pre and post menopausal females was observed.

The majority of the breast carcinoma was of the ductal type (93.4%) and there was no association between age and pathological type of carcinoma (non tabulated data).

Table 4 - Distribution of breast cancer cases and controls according to reproductive history

Reproductive history	Controls n=153	Cases n=198	p value	OR (95% CI)
Age at menarche				
Mean±SD	11.5 ± 4.7	10.6 ± 5.1	>0.05	
History of irregular menses: N (%)	25 (16.3)	30 (15.2)	>0.05	
Time of irregularity: N (%)	n=23	n=30		
• At start of menarche	0	6 (20.0)	0.014*	0.52 (0.39 to 0.68)
• All life	9 (39.1)	7 (23.3)	>0.05	
Duration of menstrual cycle (days)	n=150	n=193		
Mean±SD	30.1 ± 10.3	28.5 ± 8.5	>0.05	
Duration of menstruation (days)	n=150	n=194		
Mean±SD	5.0 ± 1.8	5.1 ± 2.2	>0.05	
Menopausal status: N (%)				
• Postmenopausal	70 (45.8)	87 (43.9)	>0.05	
• Premenopausal	83 (54.2)	111 (56.1)		
Age at menopause (years)	n=70	n=87		
Mean±SD	46.4 ± 5.9	46.7 ± 5.9	>0.05	
Causes of menopause: N (%)	n=70	n=87		
• Age	47 (67.1)	65 (74.7)		
• Surgery	12 (17.1)	6 (6.9)	>0.05	
• Medicine	4 (5.7)	10 (11.5)		
• Others	5 (7.1)	4 (4.6)		
Age at first pregnancy (years)				
Mean±SD	20.7 ± 4.6	22.5 ± 5.1	>0.05	
Age at last pregnancy (years)				
Mean±SD	32.9 ± 5.1	32.8 ± 5.3	>0.05	
Breast feeding: N (%)	n=145	n=188		
	135 (93.1)	178 (94.7)	>0.05	
Duration of breast feeding (months)				
Mean±SD	75.5 ± 21.8	75.4 ± 24.2	>0.05	

* Statistically significant

The distribution of estrogen and progesterone receptors (ER and PR respectively) among different age groups was studied. The majority of the cases (46%) showed moderate immunoreaction with ER; the 40.5% of cases showed mild immunoreaction, the minority of cases (13.2%) showed marked immunoreaction. Regarding PR, the majority of the studied cases (61.7%) showed mild immunoreaction, the 27% of cases showed mild immunoreaction and the minority of cases (11.3%) showed marked immunoreaction. Both ER and PR percentage increase as the age group but no statistically significant difference was observed (non tabulated).

Table 8 shows the distribution of ER and PR according to TNM classification; most of the cases showed no metastases. PR was mostly expressed

Table 5 - Types , indication and duration of intake of reproductive hormones in cases versus controls

Intake of reproductive hormones	Controls n=153	Cases n=198	p value	OR (95% CI)
Ever use of hormonal contraception: N (%)	72 (47.1)	107 (54.0)	0.194	1.3 (0.9-2.0)
Duration of intake of hormonal contraception[#]: N (%)	n=67	n=82		
• < 5years	30 (44.8)	43 (52.4)	>0.05	
• ≥ 5 years	37 (55.2)	39 (47.6)		
Use of therapeutic hormones: N (%)	16 (10.5)	32 (16.2)	0.123	1.6 (0.9-3.1)
Indication for hormonal intake: N (%)				
• Menstrual irregularities	5 (3.3)	4 (2.0)	>0.05	
• Menorrhagia	0	4 (2.0)	>0.05	
• Post hysterectomy	1 (0.7)	6 (3.0)	>0.05	
• Postmenopausal symptoms	1 (0.7)	0	>0.05	
• Postmenopausal osteoporosis	0	1 (0.5)	>0.05	
• Galactorrhea	2 (1.3)	10 (5.1)	0.049*	4.0 (0.9-18.6)
Duration of hormonal treatment Median (IQR)**	9 (3.0-53.0)	10 (3.2-60.0)	>0.05	
Range of hormonal treatment for menstrual irregularities (months)	3-24	3-120		
Ever use of hormone replacement therapy (HRT)	1 (0.7)	3 (1.5)	0.451	
Ever use of exogenous hormones[§]: N (%)	81 (52.9)	120 (60.6)	0.150	1.4 (0.9-2.1)

[#] some missing data
** IQR = inter quartile range
[§] Ever use of exogenous hormones was considered for intake of hormonal contraception, in addition to intake of therapeutic hormones and Hormonal Replacement Therapy
* Statistically significant

among the group having no lymph nodes and expression increases as the tumour classification.

Table 9 shows the association of intake of hormones and TNM classification among breast cancer cases. No statistically significant difference was observed in the TNM classification of breast cancer between cases with history of intake of reproductive hormones and those who did not take hormones.

Discussion

This study was carried out to investigate mainly the association between breast cancer and history of intake of exogenous reproductive hormones (estrogens and progesterone) and identify other known risk factors.

The present study revealed that the mean age for cases was 46.3±8.3. Similar mean age of cases was reported in Elattar study, based on 10,556 cancer cases at Egypt National Cancer Institute, with the median age for breast cancer of 47 years (15). Khatib and Modjtabai also stated that in Egypt, Bahrain, Jordan, Kuwait, Lebanon, Oman, Saudi Arabia and Tunisia, breast cancer is more commonly diagnosed in women under the age of 50 years, unlike the USA, where women aged 50 years and older are the most commonly affected (16). Most of the cases who attend these hospitals are from lower socioeconomic class with low educational level, both for cases and controls, as we expected.

Some of the previously known risk factors for breast cancer were not identified.

In the present study, exposure to current or past

Table 6 - Mean endogenous estrogen and progesterone serum levels in relation to premenopausal and postmenopausal females among cases and controls[a]

Variables	Premenopausal females		p value
	Controls n=67	Cases n=63	
Progesterone			
Follicular phase	n=26	n=24	
Mean ±SD	1.18±2.47	2.8±8.4	0.05
Luteal Phase	n=41	n=39	
Mean ±SD	3.3±3.9	4.6±9.4	0.09
Estrogen			
Follicular phase	n=26	n=24	
Mean ±SD	84.5±50.6	83.2±88.6	0.139
Luteal phase	n=41	n=39	0.066
Mean ±SD	94.6±66.7	105±85.4	
Variables	Postmenopausal females		P value
	Controls	Cases	
Progesterone	n= 66	n=83	
Mean ±SD	0.95±4.6	1.0±2.9	0.054
Estrogen			
Mean ±SD	30.7±31.5	26.4±18.9	0.72

[a]Hormonal serum levels were measured only for 146 cases and 133 controls

passive smoking at home was significantly higher among the breast cancer cases (p<0.001). Recent research has found passive smoke exposure to be associated with increased breast cancer risk, on the basis of an antiestrogenic effect of smoking (17). More than 30 carcinogenic chemicals are present in tobacco smoke; many of them are fat-soluble, resistant to metabolism and can be stored in breast adipose tissue (18). The result of our present study agrees with the conclusion of the California Environmental Protection Agency (Cal/EPA) report on environmental tobacco smoke (ETS) and breast cancer which is based on a systematic review of 15 studies with a significantly increased risk of 1.40 (95% CI: 1.17-1.68) that was stronger in premenopausal women (OR=2.20) (19). Furthermore, Morabia concluded that the strength of the association with breast cancer is similar for passive as for active smoking (20). This result also agrees with the researchers of Public Health Agency of Canada who examined the link between passive smoking and breast cancer from 20 published studies. They found that long-term exposure to passive smoking was associated with a 27% increased cancer risk among women who were lifetime non-smokers (21). In contrast, a case control study in the UK found no evidence of an association between either active smoking or passive smoking in the home and risk of breast cancer (22). Other studies showed no significant increased risk for breast cancer for ETS exposure in childhood or the workplace or from the spouse specifically, but an increase was seen for total exposure (23).

The present study revealed that breast cancer cases were more exposed to multiple environmental exposures than controls as insecticides (p<0.05). Evidence regarding organochlorine exposure and breast cancer risk is mixed, where not all of them associated (24).There are many mechanisms by which environmental chemicals (including tobacco smoke) and pesticides have a role in breast cancer risk as illustrated in the review of Mukherjee and his collegues (18). However, others state that involvement of pesticides in breast cancer has not yet been determined and developing countries lack sufficient epidemiologic research and evidence linking pesticide exposure with cancer development (25).

Estrogens are closely related to the pathogenesis of breast cancer. Long duration of estrogen and progesterone are associated with increased breast cancer risk, while short duration of pregnancy level doses are associated with a reduced breast cancer risk (26). Oxidative catabolism of estrogens, mediated by various cytochrome P450 enzymes, generates reactive free radicals that can cause oxidative damage. Similarly, pesticides are also known to cause oxidative stress; while some act as endocrine disruptor, some are shown to suppress apoptosis in estrogen sensitive cell lines. Many of the environmental pollutants suppress the immunitary system, which are implicated to risk (18).

The present study showed that the breast cancer cases had more menstrual irregularity early at the age of menarche, and there was no significant difference regarding age of menarche between cases and controls. Menstrual and reproductive history and postmenopausal hormone use are well-established risk factors for breast cancer (27). Early age at

Table 7 - Distribution of breast cancer cases and controls according to anthropometric measurements in pre and postmenopausal women

Groups	Variables	Controls N (%)	Cases N (%)	p value
Overall	Body Mass Index Grade:	n=149	n=181	
	• I: Normal	16 (10.7)	10 (5.5)	
	• II: Overweight	37 (24.8)	47 (26.0)	>0.05
	• III: Obesity	83 (55.7)	93 (51.4)	
	• IV: Massive obesity	13 (8.7)	31 (17.1)	
	Waist/Hip Ratio Grades	n=151	n=188	
	• < 0.8	8 (5.3)	12 (6.4)	>0.05
	• ≥ 0.8	143 (94.7)	176 (93.6)	
Premenopausal	Body Mass Index Grade:	n=79	n=98	
	• I: Normal	10 (12.7)	6 (6.1)	
	• II: Overweight	18 (22.8)	22 (22.4)	>0.05
	• III: Obesity	44 (55.7)	56 (57.1)	
	• IV: Massive obesity	7 (8.9)	0	
	Waist/Hip Ratio Grades	n=81	n=105	
	• < 0.8	7 (8.6)	10 (9.5)	
	• ≥ 0.8	74 (91.4)	95 (90.5)	>0.05
Postmenopausal	Body Mass Index Grade:	n=70	n=83	
	• I: Normal	6 (8.6)	4 (4.8)	
	• II: Overweight	19 (27.1)	25 (30.1)	>0.05
	• III: Obesity	39 (55.7)	37 (44.6)	
	• IV: Massive Obesity	6 (8.6)	17 (20.5)	
	Waist/Hip Ratio Grades	n=70	n=83	
	• < 0.8	1 (1.4)	2 (2.4)	>0.05
	• ≥ 0.8	69 (98.6)	81 (97.6)	

menarche was significantly associated with breast cancer risk in several previous studies, but was not revealed in the present study (27-30). Previous studies that have assessed menstrual factors in association with risk of benign proliferative epithelial disorders (BPED) of the breast, putative precursors of breast cancer, have yielded inconsistent findings (31). Previous studies revealed different findings than our study as no clear relationship was seen between menstrual irregularities and breast cancer risk, moreover, a negative association was previously reported where women having lifelong menstrual irregularities were at significantly reduced risk of breast cancer compared to those reporting regular cycles (27, 32), More years of menstruation and more years of menstrual before giving first birth were significantly associated with breast cancer in a previous study (29). Tamakoshi and his collegues showed there was no association between age at menarche and breast cancer risk similar to our present study (33).

In a hospital based case control study in Alexandria Main University Hospital, there was no statistically significant difference between cases and controls regarding menopausal status, menstruation span, number of births, breast feeding and use of oral contraceptives in accordance with the present study (30).

The present study did not reveal an association between duration of breast feeding and breast cancer risk. Long duration of breast feeding was previously reported as a protective factor against breast cancer (34, 35).

Table 8 - Estrogen and progesterone receptors and TNM classification

	Estrogen Receptors		Progesterone Receptors	
	Absent	Present	Absent	Present
TNM (N): N (%)				
• N0	12 (26.7)	33 (73.3)	11 (24.4)**	34 (75.6)
• N1	9 (21.4)	33 (78.6)	11 (26.2)	31 (73.8)
• N2	32 (36.8)	55 (63.2)	37 (42.5)	50 (57.5)
TNM (T): N (%)				
• T1	9 (26.5)	25 (73.5)	12 (35.3)	22 (64.7)
• T2	31 (28.7)	77 (71.3)	33 (30.6)	75 (69.7)*
• T3	11 (39.3)	17 (60.7)	11 (39.3)	17 (60.7)
• T4	2 (66.7)	1 (33.3)	3 (100.0)	0

* p=0.06; **p=0.025

Regarding hormonal treatment, no significant difference was observed among cases and controls as regards the intake of hormonal contraception, the mean duration of intake, and the intake of HRT, but the breast cancer cases showed significantly longer mean duration of hormonal treatment for menstrual irregularity ($p<0.05$).

Most studies found no or weak association of oral contraceptive pills use with the risk of breast cancer which agrees with our finding (6, 7).

As regards HRT and its relation with breast cancer risk, only 3 cases and 1 control reported used of HRT in the present study and no statistically significant difference was observed among cases and controls. Same finding was revealed with Norsa'adah and his colleague study (36). Our findings were contradictive to Li and his colleagues who found that ever users of combined HRT had a 1.7-fold increased risk of breast cancer (37). However, a significantly negative correlation between HRT use and breast cancer was revealed among Japanese women by Saeki and his collegues (38). As the use of HRT was very few in our study so we can not prove or disprove the association between HRT and cancer breast.

The relationship between clinically apparent breast cancer and postmenopausal HT has been clarified in the last few years. Most women using HT for short-term treatment of vasomotor symptoms would not face measurable increases in their risk for breast cancer. That risk returns to baseline few years after discontinuation of hormones (12). Recent studies show that there is a clinically and statistically significant increased risk of a new breast cancer event in women who took HT (31, 39, 40).

The present study showed no statistically significant difference between cases and controls as regards the BMI grades and WHR. No difference was observed among pre and post-menopausal women as regards anthropometric measures. Tehard and his colleagues reported similar results among post-menopausal women where no significant positive trends in breast cancer risk occurred with increasing weight and BMI, while among premenopausal women, weight and BMI were inversely related to breast cancer risk (41). Other studies showed an association among the postmenopausal women and breast cancer risk (29, 34, 42, 43). The explanation of a reduced risk of breast cancer among premenopausal women with BMI and other measures of adiposity is likely to result from an increased frequency of anovulatory cycles that lead to lower levels of serum estradiol and progesterone among young obese women. In postmenopausal women, estrogen production is directly correlated with body weight (44).

The pathological findings of most breast cancer patients in the present study were of the ductal type. This agrees with Omar and his collegues study in Egypt as the majority of breast tumours were invasive duct subtype and the profile of hormone receptors is positive for ER and/or PR in less than half of cases (2). Common genetic variants can influence the pathologic subtype of breast cancer, and provide support for the hypothesis that ER+ and ER- disease result from different etiologic pathways Garcia-Closas and Chanock (45). The limited number of

Table 9 - Association of intake of hormones and TNM classification among breast cancer cases

TNM Classification	History of hormonal intake		p value
	Absent N (%)	Present N (%)	
Metastasis (M):			
• No Metastasis	103 (99.0)	71 (100.0)	>0.05
• Metastasis	1 (1.0)	0	
Node (N) *:			
• N0	24 (23.1)	21 (29.6)	
• N1	26 (25.0)	16 (22.5)	>0.05
• N2	54 (51.9)	34 (47.9)	
Tumour (T):			
According to size (T)**			
• T1	20 (19.4)	14 (19.7)	
• T2	67 (65.0)	42 (59.2)	>0.05
• T3	14 (13.6)	14 (19.7)	
• T4	2 (1.9)	1 (1.4)	
• Special Type	14 (12.7)	7 (8.7)	>0.05
• No Special Type	89 (87.3)	63 (91.3)	
Types of Tumour:			
• Tubular	2 (1.9)	1 (1.4)	
• Medullary	1 (1.0)	0	
• Mucoid	2 (1.9)	1 (1.4)	>0.05
• Papillary	0	1 (1.4)	
• Classic Lobular	7 (6.7)	2 (2.8)	
• Pajet's Disease	2 (1.9)	2 (2.8)	
Estrogen Receptors			
• Positive	52 (68.4)	69 (69.7)	>0.05
• Negative	24 (31.6)	30 (30.3)	
Progesterone Receptors			
• Positive	46 (60.5)	69 (69.7)	>0.05
• Negative	30 (39.5)	30 (30.3)	

* (N0) No Node, (N1) Mobile Nodes, (N2) Fixed Nodes
** (T4) Involving Chest Wall/ Skin,

cases in some histological types of breast tumors in the present study didn't enable analyses of this relation, however previous sudies provide some insight into the different etiologies of various histologic subtypes of breast cancer (46).

On applying the TNM classification, the study showed that most of the cases had no metastases, which could be due to rapid seeking for medical advice PR were expressed most among the group having no lymph nodes and expression increases as the tumour classification.

Regarding the TNM classification of breast cancer in the present study no statistically significant difference was found between cases with history of intake, of reproductive hormones and those with no history of intake, and also regarding the type of tumour. The limited use of HRT among women in the present study did not allow accurate investigation of this association, so further studies are needed. A significant interaction between HRT use and tumour hormone receptor status on risk of recurrence was previously reported by Brewster and his colleagues.

This study indicates that the biology of hormone receptor-positive disease in HRT users differs from that in nonusers (47).

In conclusion, among all the studied epidemiological variables, passive smoking (current and past), history of breast trauma, and living near a factory were the significant factors associated with breast cancer. Both ER and PR were more expressed in the study group, who have ever taken estrogen or progesterone, but the association was not statistically significant. No statistically significant difference was observed among cases and controls as regards the history of intake of exogenous reproductive hormones (estrogen and progesterone) among the studied females.

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References

1. MOHP. Egyptian Health Information System, Ministry of Health and Population: Half Annual Report, 2002.
2. Omar S, Khaled H, Gaafar R, *et al*. Breast cancer in Egypt: a review of disease presentation and detection strategies. East Mediterr Health J 2003; 9 (3): 448-63.
3. Bedwani R, Abdel-Fattah M, El-Shazly M, *et al*. Profile of familial breast cancer in Alexandria, Egypt. Anticancer Res 2001; 21 (4B): 3011-4.
4. Boulos S, Gadallah M, Neguib S, *et al*. Breast screening in the emerging world: high prevalence of breast cancer in Cairo. Breast 2005; 14 (5): 340-6.
5. Mc Kelvey KD Jr, Evans JP. Cancer genetics in primary care. J Nutr 2003; 133 (11 Suppl 1): 3767S-72S.
6. Colditz GA. Relationship between estrogen levels, use of hormone replacement therapy and breast cancer. J Natl Cancer Inst 1998; 90 (11): 814-23.
7. Ross RK, Paganini-Hill A, Wan PC, *et al*. Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. J Natl Cancer Inst 2000; 92 (4): 328-32.
8. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of individual data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet 1997; 350 (9084): 1047-59.
9. Vandenbroucke JP, van Leeuwen FE, Helmerhorst FM. Breast cancer and the use of the hormones around the menopause. Ned Tijdschr Geneeskd 2003; 147(38): 1829-34.
10. Rosenberg L, Zhang Y, Coogan PF, *et al*. A case-control study of oral contraceptive use and incident breast cancer. Am J Epidemiol 2009; 169 (4): 473-9.
11. Chen FP. Postmenopausal hormone therapy and risk of breast cancer. Chang Gung Med J 2009; 32 (2): 140-7.
12. Nelson AL. Postmenopausal hormone therapy and breast cancer: where do we stand? Int J Fertil Womens Med 2003; 48 (5): 206-16.
13. Bray G.A. The underlying basis for obesity: relationship to cancer. J Nutr 2002; 132: 3451S-5S.
14. Baumgartner KB, Hunt WC, Baumgartner RN, *et al*. Association of body composition and weight history with breast cancer prognostic markers: divergent pattern for hispanic and non-hispanic white women. Am J Epidemiol 2004; 160 (11): 1087-95.
15. Elatar I. Cancer registration, NCI Egypt 2001. Cairo, Egypt, National Cancer Institute, 2002; http://www.nci.edu.eg/Journal/nci2001%20.pdf.
16. Khatib OMN, Modjtabai AA. EMRO technical publications series 30, Guidelines for the early detection and screening of breast cancer, World Health Organization, Epidemiology of breast cancer; 2006; 11-3.
17. Coyle YM. The effect of environment on breast cancer risk. Breast Cancer Res Treat 2004; 84 (3): 273-8.
18. Mukherjee S, Koner BC, Ray S, *et al*. Environmental contaminants in pathogenesis of breast cancer. Indian J Exp Biol 2006; 44 (8): 597-617.
19. Elwood JM, Burton RC. Passive smoking and breast cancer: is the evidence for cause now convincing? MJA 2004; 181 (5): 236-7.
20. Morabia A. Smoking (active and passive) and breast cancer: epidemiologic evidence up to June 2001. Environ Mol Mutagen 2002; 39: 89-95.
21. Irish health, 2005: Breast cancer, passive smoking linked. http://www.irishhealth.com/index.html?level=4&id=7634&ss=passive%20smoking%20and%20breast%20cancer.
22. Roddam AW, Pirie K, Pike MC, *et al*. Active and passive smoking and the risk of breast cancer in women aged 36-45 years: a population based case-control study in the UK. Br J Cancer 2007; 97 (3): 434-9.
23. Lee PM, Hamling J. Environmental tobacco smoke exposure and risk of breast cancer in nonsmoking women: a review with meta-analyses. Inhal Toxicol 2006; 18 (14): 1053-70.

24. Mitra AK, Faruque FS, Avis AL. Breast cancer and environmental risks: where is the link? J Environ Health 2004; 66 (7): 24-32.
25. Jaga K, Dharmani C. The epidemiology of pesticide exposure and cancer: a review. Rev Environ Health 2005; 20 (1): 15-38.
26. Medina D. Mammary developmental fate and breast cancer risk. Endocr Relat Cancer 2005; 12 (3): 483-95.
27. Kvåle G, Heuch I. Menstrual factors and breast cancer risk. Cancer 1988; 62 (8): 1625-31.
28. Gao YT, Shu XO, Dai Q, *et al*. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. Int J Cancer 2000; 87 (2): 295-300.
29. Han DF, Ma J, Zhou X, *et al*. A case-control study on the risk of female breast cancer in Wuhan area. Zhonghua Liu Xing Bing Xue Za Zhi 2004; 25 (3): 256-60.
30. Kishk NA. Breast cancer in relation to some reproductive factors. J Egypt Public Health Assoc 1999; 74 (5-6): 547-66.
31. Cui Y, Page DL, Lane DS, *et al*. Menstrual and reproductive history, postmenopausal hormone use, and risk of benign proliferative epithelial disorders of the breast: a cohort study. Breast Cancer Res Treat 2009 Mar 22. [Epub ahead of print].
32. Parazzini F, La Vecchia C, Negri E, *et al.* Lifelong menstrual pattern and risk of breast cancer. Oncology 1993; 50 (4): 222-5.
33. Tamakoshi K, Yatsuya H, Wakai K, *et al*. Impact of menstrual and reproductive factors on breast cancer risk in Japan: Results of the JACC study. Cancer Science 2005; 96: 57.
34. Naieni KH, Ardalan A, Mahmoodi M, *et al*. Risk factors of breast cancer in north of Iran: a case-control in Mazandaran Province Asian Pac. J Cancer Prev 2007; 8 (3): 395-8.
35. Shema L, Ore L, Ben-Shachar M, *et al*. The association between breastfeeding and breast cancer occurrence among Israeli Jewish women: a case control study. J Cancer Res Clin Oncol 2007; 133 (8): 539-46.
36. Norsa'adah B, Rusli BN, Imran AK, *et al*. Risk factors of breast cancer in women in Kelantan, Malaysia, Singapore. Med J 2005; 46 (12): 698.
37. Li CI, Malone KE, Porter PL, *et al*. Relationship between long durations and different regimens of hormone therapy and risk of breast cancer. JAMA; 2003 289 (24): 3254-63.
38. Saeki T, Sano M, Komoike Y, *et al*. No increase of breast cancer incidence in Japanese women who received hormone replacement therapy: overview of a case-control study of breast cancer risk in Japan. Int J Clin Oncol 2008; 13 (3): 279.
39. Holmberg L, Iversen OE, Rudenstam CM, *et al*. Increased risk of recurrence after hormone replacement therapy in breast cancer survivors. J Natl Cancer Inst 2008; 100 (7): 475-82.
40. Newcomb PA, Egan KM, Trentham-Dietz A, *et al*. Prediagnostic use of hormone therapy and mortality after breast cancer. Cancer Epidemiol Biomarkers Prev 2008; 17 (4): 864-71.
41. Tehard B, Clavel-Chapelon F. Several anthropometric measurements and breast cancer risk: results of the E3N cohort study. Int J Obesity 2006; 30: 156-63.
42. Safi El-Dine Ashwak M, Abdel Salam Mostafa A, El-Bagoury Iman MS, *et al*. Anthropometry and breast cancer. Egypt J Community Med 2005; 23 (3): 49-62.
43. Abdel-Hafez AM. The role of HDL-Cholesterol in breast cancer, a case control study, thesis for MD degree, Faculty of Medicine, Ain Shams University, 2000.
44. Feigelson HS, Jonas CR, Teras LR, *et al*. Weight gain, body mass index, hormone replacement therapy, and postmenopausal breast cancer in a large prospective study. Cancer Epidemiol Biomarkers Prev 2004; 13: 220-4.
45. Garcia-Closas M, Chanock S. Genetic susceptibility loci for breast cancer by estrogen receptor status. Clin Cancer Res 2008; 14 (24): 8000-9.
46. Li CI, Daling JR, Malone KE, *et al*. Relationship between established breast cancer risk factors and risk of seven different histologic types of invasive breast cancer. Cancer Epidemiol Biomarkers Prev 2006; 15 (5): 946-54.
47. Brewster AM, Do KA, Thompson PA, *et al*. Relation between epidemiologic risk factors and breast cancer reccurrence. J Clin Oncol 2007; 25 (28): 4438-44.