URBAN-RURAL DIFFERENCES OF FEMALE CANCERS IN GHARBIAH, EGYPT

by

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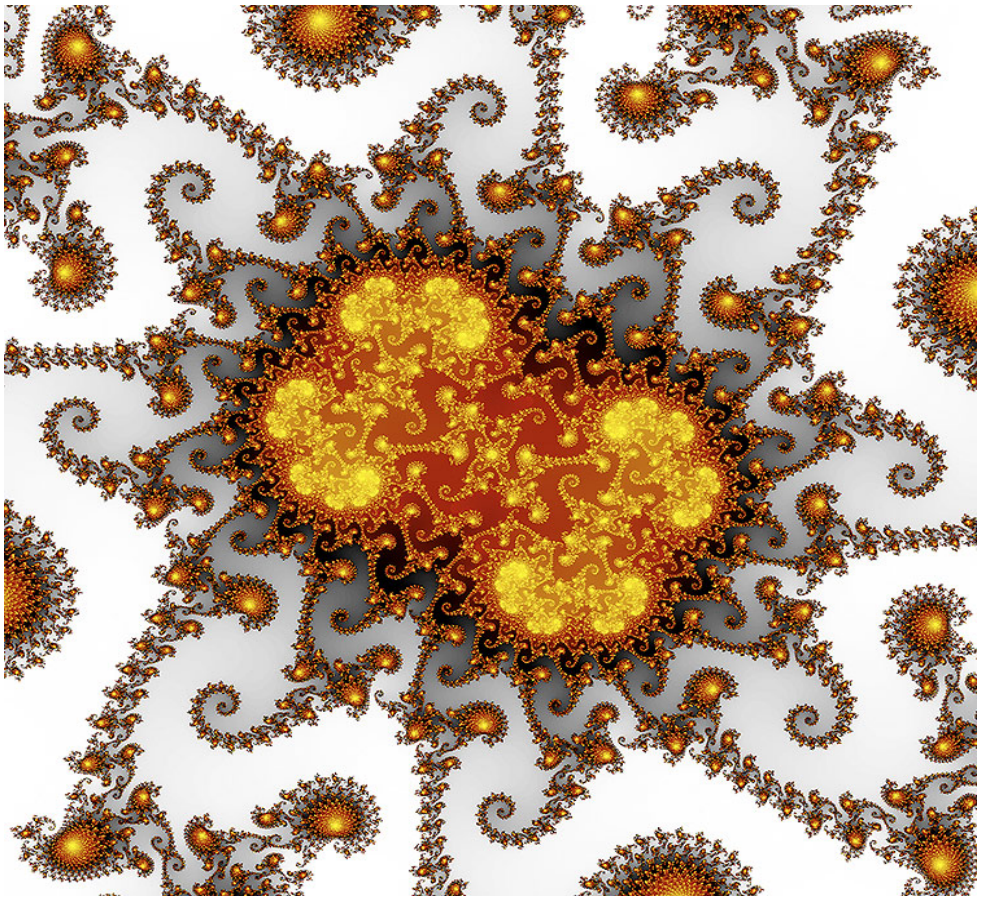

Mandelbrot set: The mathematics behind complexity.

Dedicated to

systemic thinking

where the whole is not equal to the sum of its parts

ACKNOWLEDGEMENTS

I am extremely grateful to my committee for supporting me and for providing me with valuable insights throughout this seemingly long journey of doctoral program which taught me to conduct and report quality scientific work. Time and again, I have learnt important lessons that arose from the cumulative expertise of Drs. Mark Wilson, Sofia Merajver, Joe Harford, Paolo Boffetta and Mousumi Banerjee. In particular I can probably not thank enough the role played by my mentor Dr. Amr Soliman in ensuring the success of my projects while at the same time giving me ample opportunities to exercise my creative freedom and teaching me how to conduct excellent research in imperfect circumstances. All the things that I have learnt as a part of my interactions with him and also from his being an example of a professional and exemplary scientist, will remain invaluable for me throughout my life.

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PROLOGUE

Before this I had been a doctor in India and looking at the plight of some of the patients that I saw, I realized that they required more than medical treatment. Having believed in the quote from Mahatma Gandhi – I need to be change that I want to see around me – I felt intent on deciding a course of action in my lifetime which will enable me to help maximum number of people in developing countries. Thus, I chose a path that started with enrolling into an International Health Masters program at the University of Michigan School of Public Health (UMSPH). In order to further strengthen my intellectual power I followed it with my enrollment in the doctoral program.

Along with the desire to help people I also wished to excel in research. Driven by the knowledge in paradigm shifts in scientific thought where the whole was different from the sum of its parts, I wanted to look at patterns that seemed unconnected when looked at individually but were part of the same system or whole. Hence, I decided to look at the lesser known topic of xenoestrogens and their link with breast cancer in a way that has never been approached before.

In my attempts to answer my research questions uncompromisingly, I had to travel through multiple continents, be disappointed multiple times and almost look eye to eye at the face of failure many a time before all the pieces fell into place. I was eventually able to answer, albeit in a small way, the questions that I carried with me since the day I when I had my first "Aha" moment regarding the pattern that connected xenoestrogens with breast cancer. Although it has been only three and half years, it

seems much longer to me. I hope that my work done during that time which lies ahead in ensuing pages will entice and intrigue you as much as it has intrigued me and still continues to intrigue me every day. My final goal as part of this endeavor will be to be able to contribute towards primary prevention of cancer, a seemingly difficult but achievable task.

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LIST OF ABBREVIATIONS

AJCC	American Joint Committee on Cancer
ASW	Age-standardized (to the) world (population)
BMI	Body mass index
BPA	Bisphenol - A
CA	Co-activator
CAPMAS	Central Agency for Public Mobilization and Statistics
CI	Confidence interval
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
EDC	Endocrine disrupting chemicals
EDHS	Egyptian Health and Demographic Survey
EGRF	Epidermal growth factor receptor family
ER	Estrogen receptor
ERE	Estrogen responsive elements
FFTP	First full term pregnancy
FNAC	Fine needle aspiration cytology
GPCR	Gharbiah Population-based Cancer Registry
GCS	Gharbiah Cancer Society
HCH	Hexa-chloro hexane
Her2	Human epidermal receptor2
HPV	Human papillomavirus
HR	Hormone receptor
HRS	Hormone receptor status
HRT	Hormone replacement therapy
Hsp	Heat shock protein
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IHC	Immunohistochemistry
IR	Incidence rate
IRR	Incidence rate ratio
MECC	Middle East Cancer Consortium
NAF	Nipple aspirate fluid
NDR	Nile Delta Region
NP	Nonyl-phenol
OR	Odds ratio
PABC	Pregnancy associated breast cancer
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybrominated diphenyl ether
POP	Persistent organic pollutant

PR	Progesterone receptor
PVC	Polyvinyl chloride
RR	Risk ratio
SEER	Surveillance Epidemiology and End Results
SERM	Selective estrogen receptor modulator
SNBG	Serum hormone binding globulin
TCC	Tanta Cancer Center
TDLU	Terminal duct lobular unit
TEXB	Total effective xenoestrogen burden
US	United States
WHO	World Health Organization

ABSTRACT

Breast cancer and hormone receptor positive (HR+) breast cancer incidence vary across the world with higher incidence in developed countries. Most risk factors for breast cancer are environmental and involve increased exposure to estrogen. However, known risk factors of breast cancer explain approximately 50% of breast cancer risk; thus other risk factors that are environmental, estrogenic and/or related to development might be responsible for the remnant risk. Xenoestrogens are such chemicals with wide presence in developed countries and urban areas which have been shown to increase risk of breast cancer in *in vitro* and animal studies. Human studies looking at the association of xenoestrogens with breast cancer have been equivocal. One of the reasons for this has been an absence of unexposed populations in developed countries. We hypothesized that urban incidence rates of breast cancer and HR+ breast cancer will be higher than rural rates and set out to investigate this hypothesis by comparing the two populations.

As a part of this dissertation using a series of studies which utilized data from the Gharbiah Population-based Cancer Registry (GPCR) in Egypt, we showed that urban incidence of breast cancer was three to four times higher than rural incidence. This trend held true over an eight year period for all age-groups and districts. We also showed that HR+ breast cancer incidence was two to three times higher in urban areas compared to rural areas. Further investigation into other female malignancies showed six times higher incidence of uterine cancer in urban areas than rural areas. Since both breast and uterus

are organs susceptible to estrogenic effects, a higher urban incidence of these malignancies suggested a higher exposure of urban women to estrogenic risk factors. In the absence of significant differences between urban and rural women with regards to reproductive factors, healthcare access and health behavior, differences in exposure to environmental sources of estrogens emerges as a probable factor for higher urban incidence of breast and uterine cancer.

Future studies are needed to investigate individual level differences in exposure to environmental sources of estrogen such as xenoestrogens, taking into account the presence of appropriate comparison populations in Egypt and other developing countries. Also, from these investigations we may conclude that adequate regulation of xenoestrogen use may lead to primary prevention of breast and uterine cancer.

CHAPTER I

INTRODUCTION: BACKGROUND RESEARCH

EPIDEMIOLOGY AND RISK FACTORS OF BREAST CANCER

Breast cancer is the most common malignancy among women in most developed and developing regions of the world with nearly a million new cases each year. It accounts for nearly 21% of all cancers among women worldwide [1]. The incidence rates of breast cancer are high in North America, Northern and Western Europe, intermediate in South America and Southern Europe and low in Africa and Asia [2] (Figure 1.1). The distribution of breast cancer within developing countries shows a higher incidence of breast cancer in urban than in rural areas [2], a fact that still needs to be clearly demonstrated [1, 3]. Thus, overall we see a pattern where breast cancer incidence is higher in developed parts of the world.

Among the risk factors of breast cancer, genetic causes may account for only 5-10% of breast cancer risk. Most of the risk of breast cancer is environmental in nature which has been suggested by migration studies that show an elevation of risk of postmenopausal women for breast cancer if they migrate from a low to high incidence country [4]. Some of these environmental causes are mainly related with reproductive factors which determine the exposure of women to circulating estrogens such as age of menarche, age of first full time pregnancy, number of children, breast feeding practices,

age of menopause, use of hormone replacement therapy etc. Other environmental factors that have been accounted for breast cancer include exogenous estrogens, radiation, alcohol consumption, higher education level and socio-economic status [4]. Known risk factors for breast cancer (except exogenous estrogen use, radiation exposure and alcohol consumption), are estimated to explain only 25-47% of breast cancer risk in the US [5, 6]. An overall look at risk factors of breast cancer suggests a central role of estrogen as the main causative agent in breast cancer initiation and progression. Since risk factors related to internal or endogenous sources of estrogen do not explain the entire breast cancer risk, external sources of estrogen present in the environment might be responsible for a portion of the remaining risk of breast cancer.

In addition to differences in geographical variations in incidence of breast cancer, there have also been variations in time. Incidence rates of breast cancer have been increasing in most countries, and the changes are usually greatest where the rates were previously low. There has been an approximate increase of about 0.5% annually in breast cancer incidence in the world. However, cancer registries in China are recording annual increases in incidence of over 5% which is similar to many other places in East Asia [7]. Researchers have postulated that changes in known risk factors of breast cancer might be the cause of these temporal changes in breast cancer incidence [8]. However, given the earlier estimates of the relative contributions of these factors in the causation of breast cancer, it is quite possible that relative increases in industrialization and exposure of women to environmental factors (representing external sources of estrogen) accompanying industrialization and development may have a role in the temporal increase in rates of breast cancer incidence.

BREAST DEVELOPMENT AND ENDOGENOUS HORMONES

Since hormones, especially estrogen, are such an important part of breast cancer causation, it is imperative to look deeper into how estrogen and progesterone work in the human body normally beginning with the development of the breast. In humans the female breast constitutes a network of ducts that form during intrauterine growth by branching and invasion of the mammary fat pad. These ducts are formed by a basal layer of contractile myoepithelial cells and a luminal layer of epithelial cells that line the inner duct wall. During puberty, ductal outgrowth is rapid under the influence of female sex hormones – estrogen and progesterone [9]. Estrogen leads to proliferation of cells and progesterone leads to increased differentiation [10]. During the course of normal life, the breast goes through multiple cycles of growth and apoptosis as a part of the menstrual cycle [11]. Maximum growth of breast epithelial cells is seen during the latter half of the menstrual cycle, something earlier believed to be the effect of increased progesterone levels in the luteal phase [12, 13]. However, alternative evidence shows that this may not be due to progesterone but due to delayed effect of estrogen during the first half or follicular phase of the menstrual cycle [14, 15]. The final differentiation stage in the breast is achieved during pregnancy and lactation, when numerous lobulo-acinar structures containing milk secreting alveolar cells are formed through extensive proliferation followed by terminal differentiation. Cessation of lactation after weaning leads to extensive apoptosis and tissue remodeling and the breast reverts back to its pre-pregnant state [16].

Russo *et al* [17] has clearly demonstrated the changes in internal morphology of the breast over time with breasts mainly having Lob 1 type terminal ductal lobular units

(TDLU) at the time around puberty, TDLUs being the functional component of breast and made up of an inner lining of luminal cells and outer myoepithelial cells (Figure 1.2, 1.3). With increasing age more and more of these Lob1 TDLUs get converted to Lob 2 and Lob3 type TDLUs due to more proliferation and increased differentiation. During pregnancy and lactation Lob 2 and 3 TDLUs get converted into Lob 4 TDLU and represent the maximum differentiation that can occur in the breast [17].

Apart from the role in the proliferation and differentiation of breast tissue, estrogen has been clearly implicated as a carcinogen in breast cancer due to its growth stimulatory effects [18]. These effects were indirectly inferred when George Beatson first hypothesized about the hormone dependency of breast cancer after observing regression of an advanced breast cancer following oophorectomy over 100 years ago [19]. In 1950s and 60s, radiation induced ovarian ablation was widely used for treating metastatic breast cancer which then was gradually replaced by pharmacologic therapies like tamoxifen, a selective estrogen receptor modulator (SERM) [19].

Unlike the proliferative and carcinogenic effects of estrogen however, progesterone has been found to be protective against breast cancer due to its role in causing differentiation and maturation of the epithelial cells. It is seen that progesterone levels increase mainly in the third trimester of pregnancy and this is when mammary cells undergo maximum differentiation in preparation for lactation [20, 21]. Evidence accumulated from studies looking at variation of breast cancer risk with gestation period [22, 23] and variation of breast cancer risk with progesterone levels during pregnancy [24] indeed demonstrate the protective role of progesterone in breast cancer. The only other evidence which showed progesterone to augment breast cancer was an increased

risk among women using hormone replacement therapy (HRT) with progestin and estrogen as compared to women using HRT with only estrogen. However, this increased risk may be due to the type of synthetic progestin used rather than progesterone itself [25].

HORMONE RECEPTORS

The female sex hormones act through their individual receptors belonging to the nuclear hormone family of intracellular receptors. Estrogen acts through two receptor subtypes: estrogen receptor – alpha (ERα) and ER – beta (ERβ) [26, 27] while progesterone acts through three receptor subtypes: PRA, PRB and PRC [27-29]. These receptors are mainly located on the nuclear membrane and act via affecting transcription of genes related to proliferation and differentiation. The expression of progesterone receptors (PRs) is under the control of ERα [29, 30], a fact which is quite clear from the distribution of ERs and PRs in breast tumors in most studies. Most studies show a high percentage (66%) of tumors to be ER+/PR+ (expressing both ER and PR), followed by ER-/PR- (19%). The proportion of tumors that are ER-/PR+ is the least (~2-3%) [27].

Immunohistochemistry (IHC), which is localization of antigens or proteins in tissue sections using labeled antibodies, is used for reporting of hormone receptor status. Only ERα is reported when it comes to ER status while antibodies for PRs are non-specific [31]. The reporting of ER/PR status for breast cancer has now become routine because of the accompanying significance attached to treatment recommendations. It has been observed that tumors which are positive for both receptor types respond best to anti-estrogen therapy while receptors negative for both receptor types don't show such a

response [32]. The natural history of disease between ER+ and ER- tumors also varies with better prognosis overall seen for ER+ patients. Patterns of relapse also differ with the site of recurrence more common in visceral and soft tissue for ER- cancers and in bone for ER+ cancers. The probability of recurrence is highest within the first 5 years for ER- cancers while ER+ cancers tend to relapse later [32, 33].

Epidemiological analysis of breast cancer by hormone receptor status also shows distinct patterns for ER+ and ER- cancers. Studies involving the Surveillance Epidemiology and End Results (SEER) database show ER-/PR- cancers to be more frequent before menopause [34, 35] and to be more common among African Americans than among Caucasians [36, 37]. Risk factor distribution also differs among patients based on hormone receptor status with most reproductive factors that increase a woman's lifetime exposure to endogenous estrogens resulting in ER+ breast cancer [38, 39]. Other risk factors such as genetic risks, radiation and smoking give rise to ER- breast cancers [38, 40]. Overall, these differences clearly imply that ER+ and ER- cancers denote different subtypes of breast cancer with different risk factors, clinical pictures and outcomes [19].

At the molecular level, among the ERs both ERα and ERβ have distinct distributions within the body, different modes of actions and different effects on breast cancer risk. In general, ERα is present only in about 10% of luminal epithelial cells [41] while ERβ is present in most of the cell types and is more numerous than ERα in the normal breast [42]. However, ERβ numbers steadily decrease with increase in breast neoplasia especially in proliferative lesions, carcinoma in situ and invasive disease [43]. This results in a reversal of ERα: ERβ ratio as the breast becomes neoplastic [44]. ERα

expression has also been independently implicated in increasing breast cancer risk with international studies looking at ERα having shown that its expression is higher in European than non-European women [45]. Moreover, healthy women with higher ERα expression have a higher risk of developing breast cancer [46]. It has also been seen in normal women that the levels of ERα change with levels of estrogen during a menstrual cycle with higher levels in follicular phase than in the luteal phase [47], a pattern that disappears among women with breast cancer [48].

Analysis of the mechanisms of action of these two receptor shows that ERβ works closely with genes responsible for oxidative reactions and control of reactive oxygen species and thus has a protective effect for breast cancer [49]. On the other hand, ERα is mainly the receptor that is responsible for proliferative effects in the luminal epithelial cells and is probably the most important receptor in breast cancer pathogenesis. Studies looking jointly at ERα status of cells and proliferation have also noted that cells that proliferate don't express ERs on them thus indicating the presence of a paracrine mechanism that affects their proliferation [50, 51].

Among the three progesterone receptors – PRA, PRB and PRC, the first two bind deoxyribonucleic acid (DNA) via distinct transcription activators and are present in almost equal numbers in normal breast. PRC increases the transcriptional activation of both PRA and PRB and doesn't bind DNA [28]. Expression of PRs is controlled by ERs [29, 30] and the end result of progesterone led transcription is greater differentiation of cells, one of the main reasons for progesterone being protective for breast cancer [22-24].

Apart from estrogen and progesterone receptors, other receptors also have a very important role to play in breast cancer. The chief among them seems to be the Human

epidermal receptor2 (Her2/neu) receptor also known as the Epidermal growth factor receptor family-2 (EGRF-2) which is an oncogene belonging to the epidermal growth factor receptor family [27, 30, 52]. It is an orphan receptor since it doesn't have a ligand of its own. However, it forms dimers or heteromers with other members of the same family and affects the transcription of various genes related to growth and apoptosis of cells. Its expression is low or absent in normal breast and it is seen to be overexpressed in 20-25% of all breast tumors with expression seen maximally in those tumors that lack expression of hormone receptors [52]. It is also expressed in later stages of breast cancer and in severe forms of breast cancer like pregnancy associated breast cancer and its expression indicates a very poor prognosis [27, 52].

There is a lot of cross-talk among the receptors as is depicted in Figure 1.4 [30]. This cross-talk and cross control ensures that cell growth occurs in a planned and controlled manner, a scenario just the opposite of neoplasia when this cross-talk breaks down. As is shown in Figure 1.4, ER is present on the nuclear membrane, the cytoplasm and the cell membrane. ER along with co-activator (CA) molecules controls the transcription of several genes that code for PR, pS2 and (heat shock protein) Hsp (expression is stimulated) and Her2 (expression is reduced) by acting on their promoter regions on estrogen responsive elements (ERE). However, increased expression of Her2 inhibits the activation of ERE which then reduces the expression of PR. Increased Her2 expression also increases the expression of many Hsp proteins that inhibit apoptosis and cause drug resistance to SERMs. Thus, Her2 expression signals an increase in severity of breast neoplasm with loss of control for ER, reduced cell death and drug resistance.

EXOGENOUS HORMONES

It has been mentioned in page 2 that there is probably an external source of estrogen or estrogen-like environmental compound that may be a factor in the higher breast cancer incidence in developed parts of the world. The only probable compounds that are environmental, estrogenic and related to development are xenoestrogens. P-nonyl-phenol (P-NP, a common additive in plastic) was among the first xenoestrogens to be discovered serendipitously in 1991 since it led to proliferation of breast cancer cells *in vitro* [53]. Following that, other compounds had been discovered to act like estrogens when in contact with breast cancer cells lines [54-57]. Apart from acting as estrogen, there are also a broad range of chemicals that interfere with hormonal metabolism [58].

Animal studies which followed in vitro studies showed that there was an increased risk of breast cancer among mice exposed to 4-NP compared to mice exposed to equivalent doses of estradiol [59]. A number of human studies have since then looked at various chemicals and risk of breast cancer, the common chemicals observed being dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs). Although human studies mostly have been equivocal about this association between xenoestrogens [60], better assessment methods have shown increased association between xenoestrogens and breast cancer using better assessment methods such as total effective xenoestrogen burden (TEXB) [61]. Some studies have also found credible evidence of there being gene-environment interactions between xenoestrogens like PCBs and the cytochrome enzyme system [62].

If we observe closely some of these xenoestrogens we would find that their presence is widespread since they can be found in plastics, furniture foam, detergents,

food containers, electronic equipment, cosmetics and various pesticides [63]. Among the most important chemicals are those used in plastics such as bisphenol-A (BPA) and polyvinyl chloride (PVC), pesticides and insecticides like DDTs, PCBs, aldrin, dieldrin, chlordane, lindane, methoxychlor, endosulfan, pthalates, parabens and placental extracts in cosmetics, aromatic amines and industrial solvents like benzene and toluene [63].

A number of these xenoestrogens have been found to act through the estrogen receptor. It has been shown that BPA acts through the same response pathway as natural estrogen at high as well as low doses [64-66]. Other xenoestrogens such as NP also directly stimulate ER in *in vitro* studies [67]. Studies have also reported that drugs like tamoxifen might increase the agonistic effects of xenoestrogens on mutant ERs which might have implications related to drug refractoriness and drug resistance [68]. Two studies have also demonstrated that estrogen like form of DDT increased growth of ER+ breast tumors [69, 70]. Thus, xenoestrogens might be contributing significantly to the growing incidence of not just breast cancer but ER+ breast cancer in the developed world.

BREAST CANCER IN GHARBIAH, EGYPT

As a part of this dissertation research pertaining to breast cancer, Egypt was chosen as the study site. Egypt is a North African country with a population of 78 million, most of who live around the river Nile. Almost half of the population lives in cities such as Cairo, Alexandria and cities of the Nile Delta Region (NDR) [71] which provided a good urban-rural divide to look at effects of xenoestrogen exposure. Nile, a perennial river, crosses eight countries before it enters Egypt and as such is highly polluted due to millions of

tons of pesticides washing into the river. It gets further polluted due to intensive agricultural practices in Egypt mainly involving cotton growing which has injected more than 1 million metric tons of pesticide into the environment since 1952 (beginning of pesticide evolution). This makes the NDR one of the most polluted places in the world [72]. It is estimated that almost 35-40% of Egyptian population lives in the NDR.

Egypt has a number of cancer registries most of which have been hospital based. The earliest and largest of these hospital based registries is a part of the National Cancer Institute, Cairo which receives about 1700 cases of breast cancer per year [73]. In 1998 the Gharbiah Population based Cancer Registry (GPCR) was established in Tanta (almost in the center of NDR), the capital of Gharbiah Province as a part of the Middle East Cancer Consortium (MECC). This registry records about 600 cases of breast every year from the 8 districts in the Gharbiah Province. It has a fully electronic database and uses CanReg4 software designed by International Agency for Research on Cancer (IARC) [74, 75]. We had complete access to data from this population-based registry using which we looked at urban-rural differences in the various female pregnancies, especially breast cancer.

Breast cancer rates in Egypt are intermediate when compared to rates across the world. Overall, we see an age adjusted incidence rate of 49.6 per 100,000 women. Age specific rates for breast cancer in Egypt have been compared to similar rates from the United States (US) SEER data in Table 1.1 [75]. Age-specific rates in Egypt are lower than in the US for all age-categories. In Egypt the peak incidence for breast cancer is earlier than in the US (60-64 years age category). This implies that about 62% of all

breast cancer cases in this population were diagnosed before 55 years of age as compared to only 35% of breast cancer cases in US [75].

If we look at the trends of breast cancer incidence in Egypt, only one study from the Alexandria hospital based cancer registry has shown that breast cancer incidence has been increasing (Figure 1.5). It was noted that there was an increase of about 11 folds in breast cancer incidence rate between 1972 and 2001 [76]. However, since these trends were from a hospital based registry, it was difficult to draw any conclusions about changes in breast cancer incidence in Egypt. In addition, Egyptian women have reproductive factors which include high parity and long durations of lactation [77] which when coupled with young-onset breast cancer and probable high exposure of the population to environmental sources of estrogens makes Egypt an interesting place to study breast cancer etiology.

SUMMARY OF BACKGROUND RESEARCH

Breast cancer incidence varies across the globe and has been increasing in most parts of the world as well. Most of risk factors of breast cancer are environmental and don't explain the entire risk of breast cancer. Breast undergoes numerous changes during the lifetime of a woman involving proliferation, differentiation and apoptosis. Most of these are directed by estrogen and progesterone. While progesterone has been known to be protective for breast cancer, estrogen is a known mammary carcinogen. Estrogen acts through its receptors, ERα and ERβ. While ERβ has been known to be protective for breast cancer, ERα is the main receptor (and the only reported estrogen receptor) involved in proliferation of ductal cells – the main origin of most breast cancers.

Numerous studies have also shown that risk factors for breast cancer differ according to ER status with most reproductive factors giving rise to ER+ breast cancer while other risk factors like radiation, smoking and genetics give rise to ER- breast cancer. Research based on US SEER data also shows that most of the increase in breast cancer incidence in US has been due to increase in ER+ breast cancer.

In addition to endogenous estrogens, a number of compounds in the environment have been studied in the recent decade that act as estrogens or disrupt estrogen metabolism. These compounds called xenoestrogens have been shown to cause breast cancer in animal and in vitro studies and have also been shown to increase breast cancer risk in some human studies. It has also been shown that these agents act through ER and cause ER+ breast cancers.

Finally, Egypt is an interesting place to understand the role of xenoestrogens and breast cancer since Egypt has a good population-based cancer registry in Gharbiah to which we have had access, a good urban-rural division of population with women having high parity and long durations of lactation.

SPECIFIC AIMS OF THIS DISSERTATION

Although the studies above have contributed greatly to our understanding of breast cancer, none of the studies have as of yet addressed the associations between global differences in breast cancer incidence and other lesser known environmental risk factors. With this in view the specific aims of this dissertation are:

1. Coalesce parts of the background research to formulate hypotheses which will then be used to further study the breast cancer etiology.

- This part will constitute Chapter II of this dissertation. This paper titled "Xenoestrogens may be the cause of high and increasing rates of hormone receptor positive breast cancer in the world" has been published already in *Medical Hypotheses*. (Dey S, Soliman AS, Merajver SD. Xenoestrogens may be the cause of high and increasing rates of hormone receptor positive breast cancer in the world. *Medical Hypotheses* 2009;72(6):652-6.)

2. Investigate urban-rural differences in breast cancer incidence in Egypt using data from the Gharbiah population-based cancer registry.
    - This part will constitute Chapter III of the dissertation. This paper titled "Urban-rural differences in breast cancer incidence in Egypt (1999-2006): Insights into the disease etiology" has been submitted to *Annals of Epidemiology*.
3. Examine urban-rural differences in breast cancer incidence by hormone receptor status in Egypt using data from the Gharbiah population-based cancer registry.
    - This part will constitute Chapter IV of the dissertation. This paper titled "Urban-rural differences in breast cancer incidence by hormone receptor status across 6 years in Egypt" has been accepted in *Breast Cancer Research and Treatment*.
4. Explore urban-rural differences in incidence of other female malignancies except breast cancer in Egypt using data from the Gharbiah population-based cancer registry.
    - This part will constitute Chapter V of the dissertation. This paper titled "Urban-rural differences in non-breast female malignancies in Egypt: Are

xenoestrogens involved?" is undergoing peer-review and has been submitted to The *International Journal of Gynecological Cancer*

5. Coalesce together all the finding of my dissertation to reach a conclusion.
    - This part will constitute Chapter VI of my dissertation – the final chapter.

Also present in Appendix I, is an additional paper which was the result of my data analyses using data from a multicenter breast cancer study done by IARC in South Asia. This paper lays out in detail the associations of the risk factors of breast cancer with ER status of breast cancer patients. In this mostly rural population we found a higher proportion of ER- breast cancer patients which is quite in accordance with the hypotheses explored in this dissertation. This paper is titled "Risk factors according to estrogen receptor status of breast cancer patients in Trivandrum, South India" and is In Press in *International Journal of Cancer*.

Table 1.1. Age specific incidence rates of breast cancer in Egypt compared to US SEER standardized to world population.

	Egypt	**US SEER**
Total	**49.6**	**97.2**
10-14	-	0.1
15-19	-	0.2
20-24	0.8	1.3
25-29	5.7	7.1
30-34	20.8	25.2
35-39	47.1	61.7
40-44	73.6	117.5
45-49	82.6	192.1
50-54	129.3	253.1
55-59	114.6	332.4
60-64	**134.8**	386.8
65-69	131.1	431.1
70-74	103.0	**458.7**
>75	77.6	458.7

Figure 1.1. Varied age-standardized (to the world population) incidence (per 100,000 women) of breast cancer across the world.

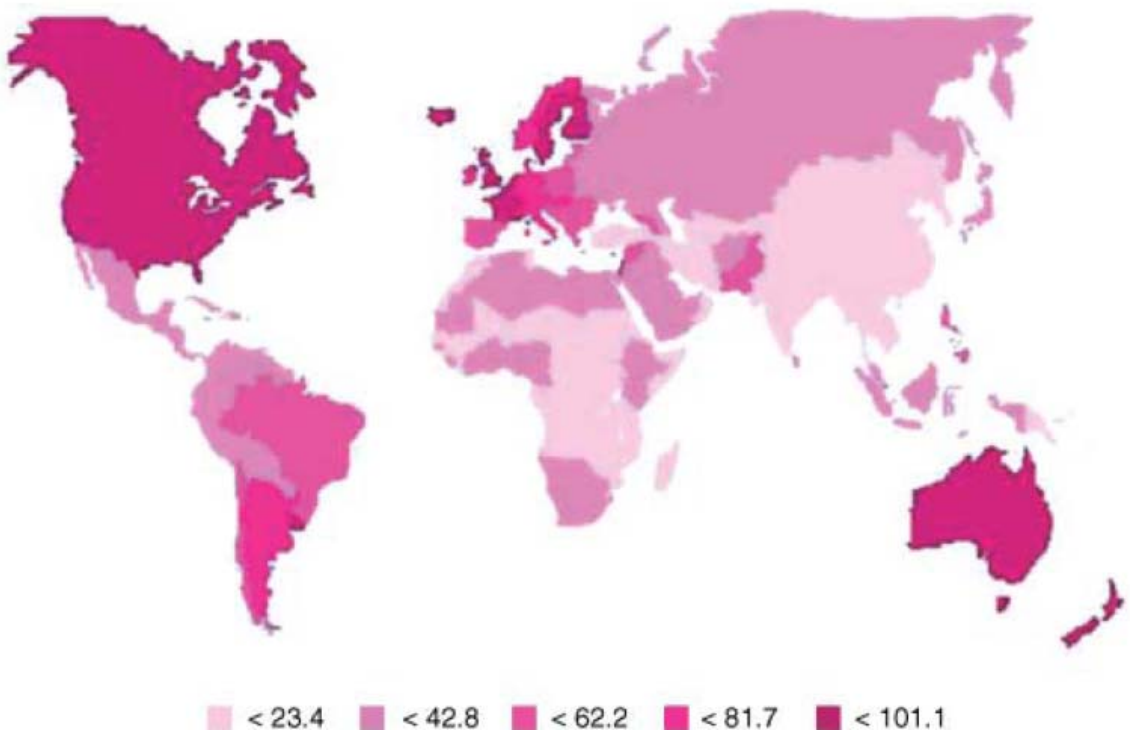
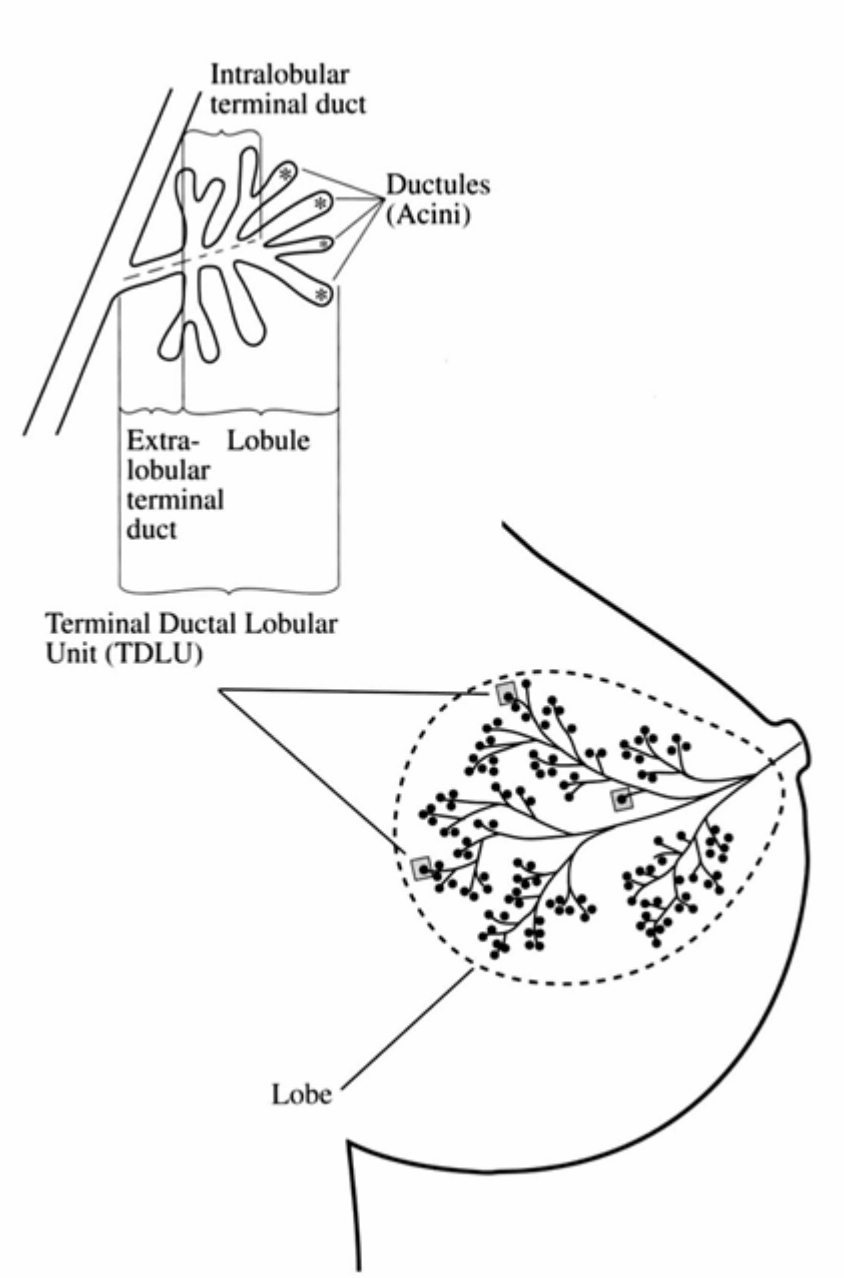
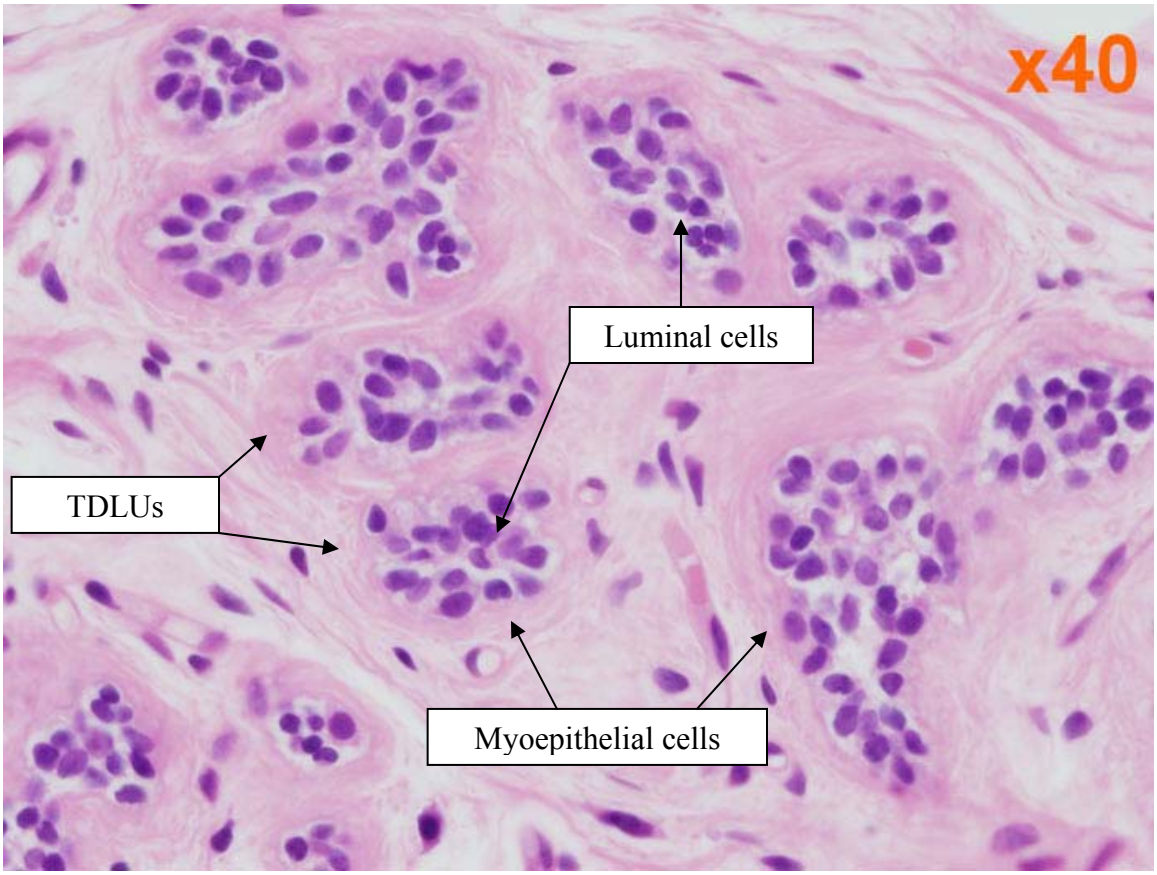


Figure 1.2. Schematic representation of TDLU.



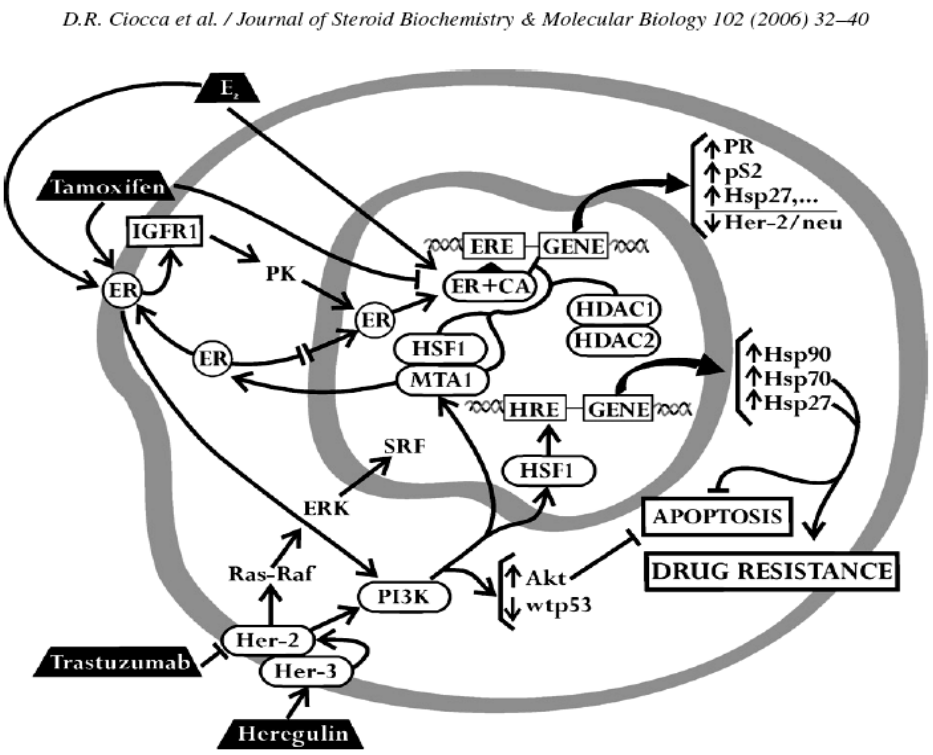
Taken from: http://herkules.oulu.fi/isbn9514270525/html/c161.html (© Tabar 1998)

Figure 1.3. Histology of a TDLU with inner luminal cells and outer myoepithelial cells.



Taken from: http://www.breastpathology.info/Sloane/lobular-neoplasia.html

Figure 1.4. Cross-talk between hormone receptors.



Acronyms in the figure: E2 = Estrogen, ER = Estrogen Receptor, PR = Progesterone receptor, CA = Co-activators, ERE = Estrogen response elements, IGFR = Insulin growth factor receptor, MTA = Metastasis associated protein, Hsp = Heat shock protein, HDAC = Histone deacetylase, HSF = Heat shock factor, HRE = Heregulin response element, Akt = Serine/threonine kinase, wtp53 = Wild type p53

Figure 1.5. Rising incidence of breast cancer in Egypt.

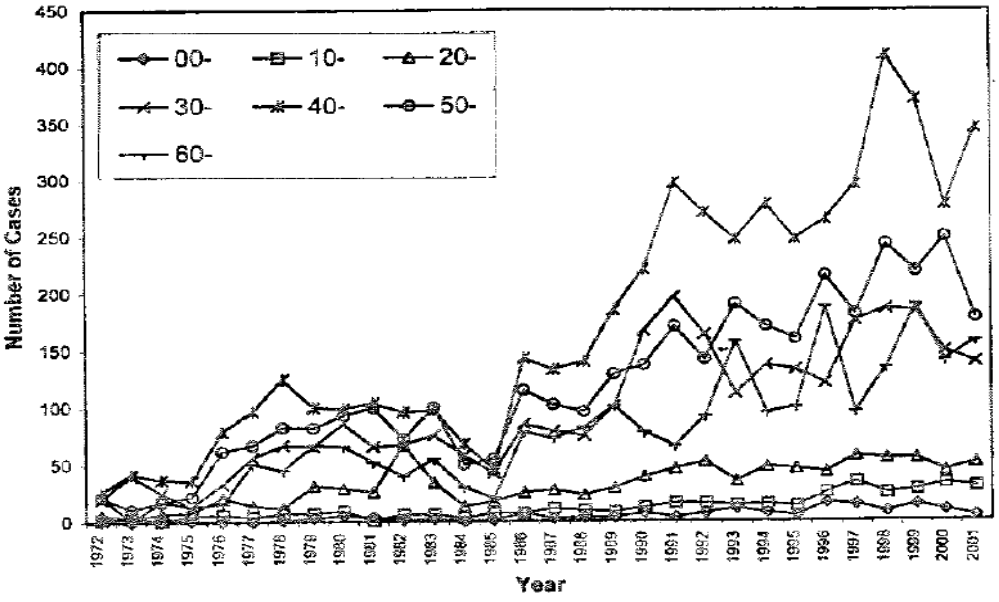


Figure 2: Female breast cancer incidence among different age-groups from 1972 to 2001. Among all age-groups, the age group of 40-50 years showed the highest incidence in breast cancer. The lowest incidence was observed in the age groups less than 25 years.

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CHAPTER II

XENOESTROGENS MAY BE THE CAUSE OF HIGH AND INCREASING RATES OF HORMONE RECEPTOR POSITIVE BREAST CANCER IN THE WORLD

INTRODUCTION

Breast cancer is the most common malignancy among women in most developed and developing regions of the world with nearly a million new cases each year. It accounts for nearly 21% of all cancers among women worldwide [1]. The incidence rates of breast cancer are high in North America, Northern, and Western Europe, intermediate in South America, and Southern Europe and low in Africa and Asia. The age-standardized (world population) incidence rates of breast cancer per 100,000 women were over 100 in Montevideo, Uruguay in South America (114.9), among Non-Hispanic Whites in California, North America (109.6) and among Hawaiians, Hawaii in Oceania (101.3). The lowest incidence rate (age-standardized) of breast cancer was seen in The Gambia in Africa (7.0) [2]. Overall, the distribution of breast cancer rates closely resembles the distribution of indicators of "affluence". Prevalence of carriers of the major susceptibility genes (BRCA1 and BRCA2) in the general population is low, and their differential distribution around the world can hardly account for much international or inter-ethnic variation in risk. Most of the differences in incident rates are therefore a

consequence of the different environmental exposures, and indeed there are quite marked changes in risk following migration, particularly if this occurs at younger age groups [3].

In addition to differences in geographical variations in incidence of breast cancer, there have also been variations in time. Incidence rates of breast cancer have been increasing in most countries, and the changes are usually greatest where the rates were previously low. There has been an approximate increase of about 0.5% annually in breast cancer incidence in the world and it has been estimated that this would result in 1.35 million new cases in 2010 [3]. However, cancer registries in China are recording annual increases in incidence of over 5% which is similar to many other places in East Asia. Still assuming a conservative estimate of 3% increase in incidence rates in east Asia, the figure for 2010 would be 1.45 million new cases, which is 82% increase on the figure in 1990 [3]. Researchers have postulated that changes in the age of childbearing, alterations in the average ages of menopause and menarche, and/or the widespread use of oral contraceptives and hormone replacement therapy might have contributed to the increasing incidence [4]. However, among the causes of breast cancer, hereditary factors account for only 5-10% of risk and the above mentioned environmental exposures account for additional 30-50% of risk [5]. Other suspected environmental factors are possibly related to the poorly-defined proportion of breast cancer risk which is also possibly the cause of temporal changes in breast cancer incidence.

In addition to differences in incidence rates, there are also differences in distributions of subtypes of breast cancer based on the estrogen receptor (ER) status and progesterone receptor (PR) expression of tumors. Based on receptor types there are four types of breast tumors: ER+/PR+ or hormone receptor positive (HR+), ER+/PR-, ER-

/PR+, ER-/PR- or hormone receptor negative (HR-). Within United States, there are differences in HR status between races with Caucasians having greater prevalence of HR+ breast cancer than other races [6, 7]. In addition, studies in other countries which have lower incidence rates of breast cancer have reported lower proportions of HR+ breast cancer [8]. It is quite possible that the higher prevalence of ER positivity may be a characteristic of populations at increased risk of breast cancer [9]. Apart from differences in space, a review of available information of ER status from US SEER has clearly shown an increase in the proportion of ER positive tumors with time while the proportion of ER negative tumors has remained constant. In fact it has been concluded that most of the increase in incidence of breast cancer in USA has been due to an increase in the incidence of ER positive breast cancer [10].

Interest in ER/PR status in the past was mainly due to differential response of patients to hormonal therapy with HR+ patients having the best response to hormonal therapy and HR- patients having the worst response [11]. However, studies soon began looking into other aspects of breast cancer based on hormone receptor status (HRS) and concluded that tumors with different HRS might in fact be different subgroups of breast cancer [12]. If we look at differences in risk factors based on HRS, it is seen that the risks associated with HR+ tumors are mostly related to reproductive factors such as nulliparity, early age of menarche and late age of menopause, use of hormone replacement therapy etc. [13, 14]. These are factors which increase a woman lifetime exposure to estrogen. On the other hand, the risk factors most closely associated with HR- tumors are independent of hormonal exposure such as smoking, family history and radiation exposures [13, 15]. Thus, this and rest of the evidence above points out that the

risk factor that is causing high and increasing risk of breast cancer across the world is probably an environmental factor that is hormonal in nature and is linked to development and affluence.

HYPOTHESIS

ER and PR are the two main hormonal receptors regulating the growth and differentiation of the breast. PR expression is under the control of ER expression [16] and progesterone is in general protective for breast cancer due to the effects of differentiation it causes in the breast tissue [17]. This implicates estrogen as the main carcinogen acting on the breast [18]. Reproductive factors have changed with development around the world with women in developed areas of the world having earlier menarche due to improved nutrition, later age of firth childbirth, lesser number of children, lesser duration of lactation, later menopause and use of hormone replacement therapy (HRT). These factors have increased the lifetime exposure of a woman to estrogen and might be responsible for an increase in HR+ breast cancer. However, estimates show that these factors along with genetic causes explain a maximum of 50% of the breast cancer risk [19, 20] which points towards other environmental factors that may have an important role to play in increasing the risk of HR+ breast cancer.

One of the probable important environmental factors that can affect breast cancer risk is chemicals present in the environment that affect the endocrine system of the body. These chemicals act like estrogen in the body or disrupt the normal metabolism of natural estrogen and thus act as carcinogens (Table 2.1) [21]. These chemicals were named endocrine disrupting chemicals (EDCs), exogenous estrogens or xenoestrogens. These

xenoestrogens (we will stick to this terminology in the rest of the paper) were serendipitously discovered by researchers in Tufts University in 1991 when they found that a chemical leaching from polystyrene tubes were causing breast cancer cells to grow in the absence of estrogen. This chemical was found to be p-nonyl-phenol, a common additive in plastics [22]. Increasing curiosity and further research by Tufts University researchers identified certain pesticides that caused breast cancer cells to proliferate in tissue cultures [23]. In another 3 years, a number of other compounds had been discovered that acted like estrogens when in contact with breast cancer cells [24-26]. Animal studies followed in vitro studies and in 2005, researchers from Texas showed an increased risk of breast cancer among mice exposed to 4-nonylphenol (4-NP) compared to mice exposed to equivalent doses of estradiol. This increased risk was due to stimulation of estriol production in liver by 4-NP [27].

In the ensuing years there have been a number of studies that have looked at circulating levels and adipose tissue levels of xenoestrogens in the body and association of these levels with breast cancer [28-30], with most of these studies finding weak associations or equivocal results [31]. One of the possible reasons for this might be the loss of power occurring from looking at associations of xenoestrogens with all breast cancer when xenoestrogens might be a possible risk factor of specific subtypes of breast cancer. Other reasons might be the presence of a number of xenoestrogens and their interaction with each other which was not taken into account [31], the use of populations where exposure to xenoestrogens is ubiquitous which reduces power due to the absence of an unexposed population or the absence of correct methods to assess xenoestrogen exposure [32]. However, better assessment methods have shown increased association

between xenoestrogens and breast cancer such as the study in Spain looking at total effective xenoestrogen burden (TEXB). This study found an increased risk of breast cancer among postmenopausal women with highest levels of TEXB-α [33]. Some studies have also found credible evidence of there being gene-environment interactions between xenoestrogens like PCBs and the cytochrome enzyme system [34].

Thus, recent evidence clearly links xenoestrogens to breast cancer and given the associations of xenoestrogen use and environmental presence with development in the modern world we hypothesize that women in developed countries and urban areas of developing countries are having increased exposures to xenoestrogens which is causing high and increasing rates of breast cancer and more specifically HR+ breast cancer. This idea as a hypothesis has not been put forth in the past. The implications of this hypothesis, if proven epidemiologically and at the molecular level, could lead to primary prevention efforts that would reduce the exposure of women to xenoestrogenic compounds and reduce the incidence of breast cancer worldwide.

EVALUATION OF HYPOTHESIS

Broadly xenoestrogens can be divided into long acting and short acting. Long acting chemicals are lipid soluble and are capable of remaining in the body for decades in conjunction with adipose tissue. Some of these chemicals, also known as persistent organic pollutant or POPs consist of a number of pesticides and insecticides such as DDT, hexa-chloro-hexane (HCH), PCBs etc [33, 35]. Being sequestered in the adipose tissue, these chemicals leach into the circulation resulting in constant minute exposure of the entire body to these chemicals over time [36, 37]. Short acting chemicals are water

soluble and are present in many articles of everyday use including plastics, bicarbonate bottles, cosmetics, food preservatives etc. Although most of these chemicals, such as bisphenol-A and parabens, are excreted out of the body rapidly, continuous exposure to them might result in constant levels of these chemicals in the body making them equally harmful.

Although there is no direct data showing the consumption patterns of xenoestrogens across the world, we can infer to their consumption based on data available for pesticide use across the world. Pesticide use in the world has been rising in the past decades mainly due to pressures on agriculture to produce more food for the growing population of the world [38]. While most of the pesticide use in developed countries has evolved on to the use of more specific products, outdated pesticides that persist in the environment are still used in developing countries. These are produced by companies in the developed world who no longer can sell them in developed countries and so continue to sell them in the developing world either through subsidiaries or local tie-ups [38].

While pesticides mostly belong to the category of long acting xenoestrogens, there has been an exponential increase in the use of short acting xenoestrogens mainly due to increasing use of plastics in all walks of life. It is estimated that almost 1 trillion plastic bags are consumed each year around the world with the US using around 380 billion plastic bags out of which approximately 100 billion are shopping bags [39]. North America and Western Europe account for 80% of plastic bag use in the world and a quarter of those bags are now made in Asia [40]. Apart from bags, plastic use is seen increasingly in all spheres of life now including but not limited to food and drink

containers, electronics, medical products etc. Short acting xenoestrogens are also seen in other categories of products such as food preservatives, cosmetics, detergents etc [41-43]. Since in the modern world use of all the above products often accompanies development, developed countries show high production and use of them in everyday life with a similar trend being seen in developing countries, mainly in urban areas.

At the molecular level, a number of these xenoestrogens have been found to act through the estrogen receptor. It has been shown that bisphenol-A (BPA) acts through the same response pathway as natural estrogen at high as well as low doses [44-46]. Other xenoestrogens such as nonylphenol also directly stimulate ER in *in vitro* studies [47]. Studies have also reported that drugs like tamoxifen might increase the agonistic effects of xenoestrogens on mutant ERs which might have implication related to drug refractoriness and drug resistance [48]. A few studies have also demonstrated that xenoestrogens like DDT and its metabolites increased growth of ER+ breast tumors with one study refuting this hypothesis [49-51].

Thus the hypothesis that xenoestrogens are the cause of high and increasing incidence of HR+ breast cancer across the world implies that xenoestrogens are related to the occurrence of a specific subtype of breast cancer. This hypothesis also predicts clearly that urban areas in developing countries would have higher incidence of breast cancer and HR+ breast cancer than rural areas. It also predicts that most of the increase in breast cancer incidence in developing countries is due to an increase in HR+ breast cancer and the urban areas of developing countries would have higher rates of increase in HR+ breast cancer than rural areas. A well planned study looking at incidence rates of breast cancer and HR+ breast cancer from a population-based cancer registry in a

developing country would be able to provide relevant supporting evidence related to the predictions emanating from the hypothesis.

DISCUSSION

Not only is exposure to xenoestrogens significant but the period in the lifetime of an individual when a woman gets exposed to estrogens or xenoestrogens is also significant [52]. If we look at the pattern of distribution of HR+ tumors by age we find from US SEER data that HR+ positive tumors tend to occur later in life compared to HR- tumors [53, 54]. Also, in developing countries where HR- tumors are more common, women tend to be younger when they develop breast cancer [55]. Further insight into when and how xenoestrogens might be acting to increase breast cancer risk comes from one of the newest areas of study in breast cancer – mammary stem cells.

Stem cells, more specifically somatic stem cells, are found in all parts of the body and are responsible for normal tissue renewal [56]. To fulfill this purpose stem cells perform asymmetric divisions in which they generate one cell identical to it and another which is more committed towards a certain differentiation pattern. Thus stem cells can maintain their population as well as produce transit cells or intermediate cells [57]. Breast is an organ that undergoes repeated cycles of growth and apoptosis throughout the lifecycle of a woman [58]. A pool of mammary stem cells has been clearly shown to be present in the breast that provides this regenerative capacity [59].

The hypothesis that cancer has its origins in stem cells is attracting a lot of attention in the scientific community [60, 61]. Presence of stem cells in many hematopoetic malignancies, solid tumors and breast tumors has further confirmed this

hypothesis [62-64]. Although a clear population of stem cells in breast is yet to be defined a putative breast tumor stem cell like population has been identified which is defined by the presence of two cell surface markers – CD44 and CD24 with these cells being CD44+/CD24- [64].

Research in humans and mice have pointed out that initial cells during early development of mammary tree do not have estrogen receptors [65, 66]. These early stem cells start out as ER- cells and differentiate to form ER+ cells post-natally that later on leads to the proliferation and differentiation of the mammary tree during puberty under the influence of estrogen [65]. A model has been proposed about cancer development from stem cells based on ER status. This model divides stem cells into three types with the most primitive cell being ER- and giving rise to ER- tumors. The intermediate stem cells have a more heterogeneous division of ERs and show limited response to SERMs and intermediate prognosis. The stem cells farthest from the primitive stem cells in this hierarchy are the ER+ progenitor cells which show maximum differentiation and good prognosis (Table 2.2) [67].

Thus it seems that risk factors that produce HR- cancer affect the primitive stem cells early in life, most likely in the intrauterine period while risk factors that produce HR+ cancer affect the intermediate or progenitor cells later in life as is also apparent from the development of HR+ tumors later in life [53, 54]. This implies that it is long term exposure to xenoestrogens throughout her life that causes an increase in HR+ breast cancer in women. At the molecular level this seems quite plausible since most long term xenoestrogens get sequestered in the adipose tissue and are released gradually into circulation [36, 37] while most short term xenoestrogens are water soluble and result in

consistent exposure in spite of constant excretion from the body. This exposes a woman to low doses of both types of xenoestrogens over time and thus increases the risk of HR+ breast cancer.

It has been known from previous studies looking at the link between xenoestrogens and breast cancer that the molecular methods to assess this link are limited and western populations might be ubiquitously exposed to xenoestrogens which makes studies inconclusive. Hence, the need for looking at xenoestrogen-breast cancer association in new populations with differential exposures and development of new molecular methods for this purpose cannot be sufficiently overemphasized. That apart, these hypotheses in effect connect more closely the links between global differences in incidence of breast cancer, xenoestrogens, hormone receptor status of breast cancer and stem cells. The way in which the predictions of these hypotheses could be proven are not difficult and would add to the evidence which we seek in further defining the etiology of breast cancer. We must bear in mind that xenoestrogens are a potentially preventable putative cause of breast cancer and this association between xenoestrogens and breast cancer if confirmed should lead us to policies that ban or regulate the use of xenoestrogens. Further research into mammary stem cells can result in better treatment and intervention at the appropriate time in lifetime of a woman to reduce breast cancer incidence. In addition, monitoring efforts can be implemented that test all new manufactured chemicals for their hormonal effects and related consequent impacts on breast cancer incidence.

Table 2.1. Common chemicals linked to breast cancer.

Chemical Class	Potential Sources	Example Chemical
Pthalates	Plastic, nail polish and other cosmetics	Dibutyl pthalate
Alkylphenols	Detergent, plastic, pesticide formulations	Nonylphenol
Flame retardants	Furniture foam and stuffing, carpets and drapes, electronic equipment (TVs, computers)	Polybrominated diphenyl ether (PBDE 47)
Polycyclic aromatic hydrocarbon (PAHs)	Stoves and heaters, cigarette smoke, outdoor air pollution, auto exhaust, combustion sources such as fireplaces	Benzo(a)pyrene
Polychlorinated biphenyls (PCBs)	Older electrical equipment	PCB 52
Banned pesticides	Historical pesticide use in/near the home	DDT, dieldrin, chlordane
Currently used pesticides	Recent pesticide use in/near home	Chlopurifos, permethrin
Other phenols and miscellaneous	Disinfectants, polycarbonate plastics, cosmetics	o-phenyl phenol, bisphenol-A, parabens

Adapted from Ref. [21].

Table 2.2. Subtypes of breast cancer based on stem cell type of origin.

Cancer type	Type 1	Type 2	Type 3
Cell of origin	Stem cell	Stem cell	ER*+ progenitor
ER* expression	Negative	Heterogeneous[a]	Positive
Histology	Undifferentiated. Basal and luminal markers present,	Intermediate differentiation	Differentiated
Prognosis	Poor	Intermediate	Luminal markers only. Good.
Risk from HRT[#]	Unchanged	Limited	Increased
Efficacy of SERMs[@] in prevention	None	Limited	High
Probable period of exposure to xenoestrogens	Early in life, most likely in intrauterine period	Early to intermediate	Intermediate to late and prolonged

Abbreviations: *ER - Estrogen receptor, [#]HRT - Hormone replacement therapy, [@]SERMs - Selective estrogen receptor modifiers.
[a]ER+ cells represent 10-80%.

Adapted from Ref. [67].

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CHAPTER III

URBAN-RURAL DIFFERENCES IN BREAST CANCER INCIDENCE IN EGYPT (1999-2006): INSIGHTS INTO THE DISEASE ETIOLOGY

INTRODUCTION

Breast cancer is the most common lethal malignancy among women in most developed and developing regions of the world, with nearly a million new cases each year, accounting for nearly 21% of all cancers among women worldwide [1]. The incidence rates (IRs) of breast cancer vary worldwide, with high rates in North America, Northern and Western Europe, intermediate rates in South America and Southern Europe, and low rates in Africa and Asia [2]. Developing countries show a higher incidence of breast cancer in urban than in rural areas, a pattern that has not been fully explained [1-3]. While hereditary causes account for only 5-10% of breast cancer risk, most breast cancer risk is considered to be environmental in nature as shown by studies of women who migrate from low incidence to high incidence settings [4, 5]. Crucial risk factors for breast cancer, which modulate a woman's exposure to endogenous estrogens, such as age of menarche, age of first full time pregnancy, number of pregnancies, duration of breastfeeding, and age of menopause, only explain one-quarter to half of postmenopausal breast cancer risk in the United States (U.S.) [6, 7].

In addition to overall geographical differences, incidence of breast cancer has been increasing in most countries, particularly where rates were previously low [8]. A

recent increase of ~0.5% per year has been estimated, a trend which would produce 1.35 million new cases in 2010 [8]. While changes in recognized risk factors for breast cancer, such as shifts in reproductive habits might be contributing to such increased incidence [9], exposures of women to carcinogens associated with industrialization and other economic development factors may also play a role. Since breast cancer is incontrovertibly associated with estrogenic exposures [6, 7], and endogenous estrogens cannot explain the total non hereditary risk, it is reasonable to hypothesize that other unknown exogenous estrogenic factors may be responsible for elevated incidence in industrialized countries and incidence that appears to be increasing with development, in lower incidence areas.

Exogenous estrogens, or xenoestrogens, are environmental chemicals that mimic the action of hormones or directly affect pathways of endogenous hormones. Important among such chemicals because of their ubiquity and activity strength are those estrogen mimetics present in plastics such as bisphenol-A (BPA), phthalates and polyvinyl chloride (PVC), pesticides and insecticides like DDTs, polychlorinated biphenyls (PCBs), aldin, dieldrin, chlordane, lindane, methoxychlor, endosulfan, pthalates, parabens, and placental extracts used in cosmetics, aromatic amines, and industrial solvents like benzene and toluene [10]. At least 216 distinct potential xenoestrogenic chemicals have been evaluated by various agencies, including the US National Toxicology Program [11], or International Agency for Research on Cancer (IARC) [12], 11th Report on Carcinogens [13], Carcinogenic Potency Database [14], and Chemical Carcinogenesis Research Information System [15]. These candidate mammary carcinogens are widespread in the developed world: 73 are present in consumer products or as contaminants in food, 35 are

air pollutants, 29 are produced at a rate of over one million pounds per year in the US, and 25 are involved in cases of known hazardous occupational exposures to more than 5000 women [16].

Mounting evidence from various parts of the world shows that use and exposure to xenoestrogens accompanies development. Many studies have demonstrated that various xenoestrogens are more abundant in urban areas [17-25], but widespread exposure in developed countries makes epidemiological investigation difficult [26]. Comparisons between urban and rural populations that are fairly genetically homogeneous in developing countries are ideally suited for studies of breast cancer incidence, due to their differential rates of development. Egypt is an excellent example of such a developing country with urban and rural genetically homogeneous populations that may differ in their economic engagement, resources, and environmental exposures. Urban populations are involved mainly in an industrial economy and rural populations are primarily engaged in an intensive agricultural economy [27]. Except the coastal areas and the Nile valley, 97% of land area of Egypt is desert and essentially uninhabited. The narrow tract of arable land requires intensive irrigation and agricultural practices adapted to support a rapidly growing and densely concentrated population [28]. These agricultural practices have led to heavy pollution of the Nile and extensive human exposure to various chemicals, mainly in the Nile Delta Region (NDR), which collects the drainage from all the main agricultural areas of the country, as the Nile approaches the Mediterranean Sea [29]. Public health studies have observed significant levels of residues of various pesticides on fruits, vegetables, milk, and other produce in various major markets in Egypt [29]. Progressive industrialization and urbanization of Egypt has

resulted in nearly half of the Egyptian population now living in cities [30]. Given the high exposure of urban populations to various xenoestrogens, we hypothesized that breast cancer incidence is higher in urban than in rural areas of Egypt. Accordingly, we analyzed patterns of breast cancer incidence in the Gharbiah province of Egypt, near the Nile delta, using data from the only population-based cancer registry in the country, specifically comparing urban and rural incidence rates in this Nile delta region.

METHODS

Study Population. The study population consisted of all women diagnosed with primary breast cancer during the eight years from 1999 through 2006, who were in the Gharbiah Population-Based Cancer Registry. For each case, the following information from routinely collected registry data was obtained: registry number, age at diagnosis, address, address code, smoking status, occupation, basis of diagnosis, estrogen receptor status, progesterone receptor status, tumor grade, stage, morphology, medical record number, and place of referral. Data were stripped of all personal identifiers and their analyses were approved by the University of Michigan Institutional Review Board and the Gharbiah Cancer Center Ethics Committee.

Gharbiah Population-Based Cancer Registry. The Gharbiah population-based cancer registry, founded in 1998 as a part of the Middle East Cancer Consortium (MECC) and funded by the U.S. National Cancer Institute, is located in Tanta City, the capital of Gharbiah province [31]. Through an active registration process, data on cancer cases are collected from various sources in the province. For this study, most breast cancer cases

came from three locations; the Tanta Cancer Center (40-50%), Gharbiah Cancer Society (10-12%) and Tanta University Hospital (10-12%). The remaining cases came from private pathology laboratories (10%), Mansoura University Radiotherapy and Nuclear Medicine Hospital (3-4%), government insurance hospitals (4-5%), the National Cancer Institute of Cairo University (NCI- Cairo) (2-3%), and mortality records (1-2%). Data obtained from these hospitals and centers were entered in a manner that ensured strict quality control checks and avoided repetition of cases. During 1999-2002, the registry staff visited the centers to abstract data from case files. In 2003, the registry began copying and collecting records from these hospitals and centers, followed by abstraction of data in a manner that conforms to the International Agency for Research on Cancer (IARC) software CanReg4, permitting electronic entry in the CanReg4 database. Registrars were trained in data extraction and entry methods, and they are periodically monitored by site visits from the faculty of Emory School of Public Health, IARC, and the MECC registry Steering Committee members [31].

Most of the breast cancer cases in the registry (95.8%) were diagnosed by histopathological confirmation of the primary tumor [32]. The World Health Organization (WHO) ICD-02 coding was used to determine the types of cancer from 1999-2000, followed by ICD-03 coding beginning in 2001. Cases were registered with the American Joint Committee on Cancer (AJCC) staging from 2003 onwards, while those from 1999-2002 employed the SEER staging, but were converted to AJCC staging for the purposes of this study.

Gharbiah Province. The Gharbiah province is an administrative region located 90 kilometers north of Cairo in the Nile delta region and has eight districts, each with a capital city (Figure 1). Tanta city also serves as the capital of the province. Gharbiah has a population of more than 4 million people, 49% of whom are women. Approximately 30% of the population resides in urban areas and almost 47% of the female population is below the age of 20, according to the 2006 Central Agency for Public Mobilization and Statistics (CAPMAS) national census of Egypt [33]. Most residents of rural areas are part of an agricultural economy while the majority of workers living in cities participate in industrial related and service occupations. These industries are predominantly located in the two most populous districts (Tanta and El Mehalla) [33].

Census Data. The 1996 and 2006 CAPMAS censuses were used to obtain data on women residing in Gharbiah [33], and linear regression was used to estimate the population during each study year. The linear growth rates of eight districts were applied to the urban and rural populations within those districts to determine urban and rural populations from 1999 through 2006. Twelve age categories were obtained from the census (one representing less than 24 years of age and eleven subsequent categories each comprising a 5-year interval). These population figures per age interval formed the denominators to calculate the overall, age-specific, district-specific, and urban-rural incidence rates for breast cancer in women.

Urban-Rural Classification. Urban and rural designations were made according to the CAPMAS definitions [33]. Urban areas consisted of all the capital cities of the eight

districts of the province, while the remaining areas in the province were considered rural. Each case in the registry was assigned a residence code based on their residential address that follows the CAPMAS coding. This code was used to classify cases as urban or rural.

Statistical Analysis. Descriptive statistics and rate analyses were completed using SAS (Version 9; SAS Institute, Cary, NC). Yearly raw and age-adjusted incidence rates were calculated for Gharbiah province, each of the eight districts, and urban and rural areas for the province. Crude annual incidence rates were calculated by dividing the number of cases each year by the respective population estimate for that year. Age-specific incidence rates for the entire study area and for urban vs. rural areas were calculated for each of the twelve age categories. Direct age-adjusted incidence rates were calculated by direct age-standardization for each district and their urban and rural areas using Gharbiah's 2006 population as the standard. Trends in breast cancer incidence were compared overall, and by urban-rural status, age categories and districts using negative binomial regression.

Incidence Rate Ratios (IRRs) and p-values for trend were calculated using negative binomial regression by the GENMOD procedure in SAS. Although age, histology and stage at diagnosis are potential confounders, histology was uniform in distribution across urban-rural strata and stage at diagnosis did not affect IRRs by more than 10%. Therefore, we analyzed age-standardized IRRs and 95% confidence intervals (CI). However, stage at diagnosis was a confounder for incidence trends and therefore we report the overall IRR and *P*-values for trend after adjusting for stage.

To control for known reproductive factors that may have contributed to urban-rural differences, the following formula [34] was used:

$$\mathbf{Inc_{(Urban)} \sum (n\ (Rural_j)\ RR_j / n(Rural)) / Inc_{(Rural)} \sum (n(Urban_j)\ RR_j / n(Urban))}$$

Where

$Inc_{(Urban)}$: Urban incidence rate of breast cancer

$Inc_{(Rural)}$: Rural incidence rate of breast cancer

$n(Urban_j)$ and $n\ (Rural_j)$: The number of urban and rural women respectively in the j^{th} risk factor category

n(Urban) and n(Rural): The total number of urban and rural women respectively

RR_j: The risk ratio or odds ratio (OR) associated with j^{th} risk factor category

Age-adjusted urban and rural incidences of breast cancer, ORs from an earlier Egyptian case-control study of breast cancer [35] and prevalence data from the Egyptian Health and Demographic Survey (EDHS) [36] were employed in the formula. Due to potential differences in reproductive habits and diet, we investigated age at first birth or age at first full term pregnancy (FFTP), number of children, and duration of breastfeeding as potential variables to control for urban-rural incidence difference. However, the case-control study [35] and EDHS [36] indicated that the number of children and duration of breastfeeding did not confer much risk for breast cancer, nor were these factors different for urban and rural women in Egypt. Therefore, we controlled only for age at FFTP.

RESULTS

A total of 4,794 female cases of breast cancer with an average age of 50 (± 11.4) years were identified (Table 3.1). Tanta and El Mehalla, the two largest districts of Gharbiah,

contributed the most cases, their contributions being 35.0% and 30.6% of cases, respectively. Other districts each contributed ~4 - 8% of person years (Kafr Zayat – 7.3%, Zefta – 5.9%, Samanood – 4.8%, El Santa – 5.9%, Kotour – 4.1% and Basyoon – 4.6%). Most cases were either stage 2 (33.7%) or stage 3 (45.9%) and had been diagnosed by pathological confirmation (94.4%).

Overall incidence of breast cancer in Gharbiah ranged from 30.2 per 100,000 women in 1999 to 34.5 per 100,000 women in 2006 (Table 3.2). Overall incidence of breast cancer in Gharbiah increased during the study period ($P = 0.02$) (Table 3.2). Incidence of breast cancer across the eight districts of Gharbiah was highest in Tanta and lowest in Kotour and Zefta (Table 3.3) (Figure 3.1). Age-specific breast cancer incidence rates increase in the younger age categories, peak around 45-55 years, and then decline in ages over 55 years. This pattern was consistent across the eight years of study. The age at peak incidence appears to have increased over the study years, a change consistent with an increase in overall breast cancer incidence (Figure 3.2).

Urban-rural breast cancer incidence rates showed a consistent pattern with urban rates being higher than rural rates (1999 - IRR = 4.63, 95% CI = 5.31, 4.04 and 2006 – IRR = 2.71, 95% CI = 3.83, 1.91) (Table 3.2). Overall, and throughout the eight years, the urban incidence rates were higher than rural incidence rates (Overall IRR = 3.73, 95% CI = 4.22, 3.30) (Table 3.2). Urban populations showed higher age-specific incidence of breast cancer than rural locations, for all age categories (Figure 3.3). We analyzed the difference in patterns of age-specific incidence curves: rural areas showed an age-specific breast cancer incidence pattern similar to developing countries: a rise followed by a peak and then a drop. However, the pattern for urban populations is different from the rural

pattern, with a less pronounced decrease in incidence after the peak. On adjusting for age at FFTP, we observed a reduction in urban-rural IRR by 8.9%.

DISCUSSION

This is the first study from a population-based cancer registry providing strong evidence of increasing breast cancer incidence in Egypt in the study period. Previously, a small-scale, hospital-based study from Alexandria, Egypt suggested an increase in incidence rate of breast cancer there [37]. Rising breast cancer incidence has been reported from most places in the world [38], with rapid increases observed in developing countries [39]. Although a multitude of factors may be contributing to such an increase, it is imperative to understand the role of environmental factors, both as direct causation and indirectly, by affecting other factors. Environmental influences on other risk factors are well documented, such the decreasing age at menarche that ensues with higher caloric intake; other social determinants of risk factors such as increasing age of marriage and first childbirth and shorter duration of breast feeding [40] also influence risk. Other nutritional factors such as higher meat [41] and dietary fat [42] intake, lower levels of physical activity [43], higher body mass index [44], and use of oral contraceptive pills [45] or hormone replacement therapy [46] also may be important modulators of risk.

In addition, there are study-specific factors that may lead to the observation of increased incidence that are not due to disease-specific risk factors. Since the Gharbiah registry is relatively new, it is possible that increased breast cancer incidence observed in our study was due to an increase in the number of diagnostic and treatment centers in Gharbiah [47].

It is also possible that unknown risk factors, such as xenoestrogens may have contributed to increasing breast cancer risk. Since increases in breast cancer incidence accompany development throughout the world and use/exposure to xenoestrogens is related to development and industrialization, such an association is certainly possible and can be investigated in the future. This study did not directly evaluate the role of xenoestrogens, but the urban-rural differences that were observed are consistent with further studying the hypothesis that environmental exposures may influence breast cancer risk.

We observed three-to-four times higher incidence of breast cancer in urban than in rural areas. The higher urban incidence was consistent across eight years and for all age groups. Although the above mentioned known risk factors might be responsible for the observed higher urban incidence, EDHS findings indicated that urban and rural Egyptian women had similar reproductive histories [36]. Furthermore, when we controlled for FFTP, one of the most important reproductive factors affecting breast cancer risk, the urban-rural IRR changed little. Thus, other as yet unknown risk factors, such as xenoestrogens, may be contributing to a higher urban breast cancer incidence in this region.

We considered that the elevated breast cancer incidence in urban areas relative to rural areas could be due to limited access to diagnostic facilities in rural areas, possibly causing many women in rural areas to die with breast cancer undiagnosed. However, this notion appears to not be a feasible explanation. Primary healthcare coverage in Egypt is in principle 100%, with rural areas having good access to physicians and primary care hospitals [30]. Also, rural areas in Gharbiah are no further than 50 kilometers from

Tanta, the capital city, and are mostly well-connected by readily available, inexpensive public transportation. Thus, difficulties in health care access and non-detection of cases cannot explain urban-rural or district-level differences reported in this study. EDHS results also indicate that health seeking behavior of women in northern Egypt does not differ significantly between urban and rural areas [36].

Urban and rural differences in breast cancer incidence in Egypt and other developing countries are qualitatively analogous to the pattern of differences in incidence reported between developed and developing countries. This analogy is consistent with the patterns of age-specific breast cancer incidence, where urban areas show a higher age-specific breast cancer incidence for all ages, and also age-specific incidence patterns similar to developed countries. In contrast, the lower incidence in rural areas in this study showed a decrease in incidence in later years of life, similar to that seen in developing countries [38]. We pose that the absence of a decline in incidence in older women in developed countries and urban areas could be due to sustained increased exposure to estrogenic factors throughout the lifetime. In this scenario, we further surmise that in developing countries and in rural areas, xenoestrogenic influences would be low throughout life with low accumulation of environmental risk, so that the latter half of life would fail to drive the breast cancer incidence beyond 50-60 year age-group, leading to a decline in breast cancer incidence later in life. In this proposed model, heritable factors and early life exposures would cause most of the breast cancer in developing countries and rural areas.

Breast cancer incidence between the different Gharbiah districts also varied by as much as three-folds. Since the geographic distance between an incident case's dwelling

and the registry does not appear to affect the probability that the case will be detected and, by the procedures in place to track records, it does not affect the registration, we pose that perhaps exposures related to the relative economic development and industrialization between the districts are more relevant in causing these inter-district differences. Tanta and El-Mehalla, the capitals of the respective districts, are the largest cities and are home to most of the industries and commercial centers of the province. Therefore, we speculate that women in these two districts may experience greater exposure to xenoestrogens, a hypothesis that warrants investigation.

Although the link between xenoestrogens and breast cancer has not been thoroughly explored, the evidence available so far suggests that exposure to xenoestrogens is high and increasing across the world. World pesticides sales have increased most in developing countries, at a rate which is two to three times higher than the current world average. Moreover, it is feared that pesticide exposure in developing countries may be much greater, in part due to the use of outdated and toxic pesticides, lack of technical knowledge and training, and absence of adequate equipment and safeguards [48]. Agricultural produce from rural areas is then transported to urban areas for consumption with additional pesticides being added to them for preservation during storage and transportation [49]. Of further concern, exposures to plastics, which contain BPA and phthalates — both being carcinogenic in *in vitro* and animal studies, is increasing in urban areas [50]. These compounds are being detected in the urine of people in developed countries [51-54], universally across the population. The detection of BPA and phthalates is ascribed mainly to the massive increase in plastic usage worldwide. It is estimated that almost 1 trillion plastic bags are consumed each year

around the world, with the US using around 380 billion plastic bags, out of which approximately 100 billion are shopping bags [55]. North America and Western Europe account for 80% of plastic bag use in the world and a quarter of those bags are now made in Asia [56].

Short-acting xenoestrogens are also seen in other categories of products, such as food preservatives, cosmetics, and detergents [57-59]. In addition, economic development also increasingly exposes people to air pollution arising from vehicular exhausts and industrial smoke with polycyclic aromatic hydrocarbons, which have also been implicated in breast cancer risk [16]. Taken together, the above trends which accompany development are quite prevalent in developed parts of the world and are becoming more widespread in developing parts of the world, beginning mainly in the urban areas. Recently, many studies have shown the greater presence and exposure to xenoestrogens in urban areas across many parts of the world [17-25]. In our own work in Egypt, we previously showed that urban women have higher levels of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dG) [3], suggesting that that they have had greater exposure to carcinogenic influences, probably xenoestrogens.

This study compared urban and rural populations in a developing country using the residential addresses of individuals. We classified the capital cities of the eight districts, which are the main industrial and commercial centers for each district, as urban while the villages in the district as rural. This form of classification into urban and rural is the most common one practiced by all countries across the world, following the first use of this dichotomous classification by UN in 1940 [60]. Thus, misclassification of people regarding urban-rural status is highly unlikely. Such dichotomous division of

population is increasingly complicated in areas around big cities and in developed countries [60]. However, in the context of Egypt, this dichotomous division into urban and rural still seems useful, especially in a province like Gharbiah, which has been developing only recently and does not yet have huge urban conglomerates with populations in the millions.

One of the biggest strengths of this study derives from the fact that we saw a consistent pattern across eight years between urban-rural populations, in all age categories and districts, based on a population registry. However, this study also had a few limitations. One of them might be the dichotomous classification of urban-rural status of cases based on the CAPMAS definition, which potentially may obscure other risk factors. A study in U.S. regarding how urban-rural definitions affected health outcomes concluded that: "dichotomous definitions (of urban-rural status) masked hidden heterogeneity in very rural areas when we considered an outcome that was related to health care access" [61]. However, since all the evidence from other studies points to no major barriers to healthcare access and breast cancer diagnosis in this population in Egypt, we expect that this limitation is small or negligible. Another possible limitation of our study is that 2-4% of cases were missing for the years 2003-2006, mainly from NCI Cairo and couple of pathology laboratories. In addition, looking at the dataset of 1999-2002, for which the registry is complete, it could be determined that most of these missing cases were urban (results not shown). Thus, these possible missing cases for 2003-2006 could have resulted in an underestimation of the urban-rural IRR. Also, the absence of information on some individual risk factors of breast cancer is a limitation. However, such information is not usually a part of the data collected by cancer registries,

so this limitation is not particular to our study. Other studies have shown that women living in higher socio-economic status (SES) or urban communities had increased risk of developing breast cancer that cannot be explained by their exposure to individual risk factors [62]. This provides further evidence in support of the conjecture that higher exposure to xenoestrogens in higher SES or urban communities increases breast cancer risk.

To our knowledge, no previous studies in developing countries have yet shown such a stark contrast in breast cancer incidence between urban and rural populations. Future studies looking at the association of xenoestrogens and breast cancer must consider that urban-rural populations in developing countries provide an ideal setting to analyze contrasting populations in terms of such exposures. Individual level assessment of xenoestrogen exposures in these populations may contribute to finding a possible basis for our initial findings.

Table 3.1. Descriptive information of the registry study population in Gharbiah, Egypt, 1999-2006.

Variable	Descriptive Category	Urban No. (%)	Rural No. (%)	Overall No. (%)
Total Cases		3043 (63.48)	1688 (35.21)	4794 (100)
Year of Diagnosis	1999	395 (70.54)	165 (29.46)	560 (11.68)
	2000	377 (69.69)	164 (30.31)	541 (11.29)
	2001	378 (65.51)	199 (34.49)	577 (12.04)
	2002	431 (69.40)	190 (30.60)	621 (12.95)
	2003	349 (57.69)	251 (41.49)	605 (12.62)
	2004	388 (59.06)	254 (38.66)	657 (13.71)
	2005	347 (59.22)	221 (37.71)	586 (12.22)
	2006	378 (58.42)	244 (37.71)	647 (13.50)
Age	0-24	14 (60.87)	9 (39.13)	23 (0.48)
	25-29	52 (55.91)	41 (44.09)	93 (1.94)
	30-34	143 (55.43)	115 (44.57)	258 (5.38)
	35-39	283 (57.52)	209 (42.48)	492 (10.26)
	40-44	446 (60.85)	287 (39.15)	733 (15.29)
	45-49	529 (64.99)	285 (35.01)	814 (16.98)
	50-54	542 (67.41)	262 (32.59)	804 (16.77)
	55-59	377 (65.34)	200 (34.66)	577 (12.04)
	60-64	279 (65.04)	150 (34.97)	429 (8.95)
	65-59	179 (68.32)	83 (31.68)	262 (5.47)
	70+	110 (70.06)	47 (29.94)	157 (3.28)
District*	Tanta	1213 (72.25)	466 (27.75)	1679 (35.02)
	El-Mehalla	1097 (74.88)	368 (25.12)	1465 (30.56)
	Kafr El-Zayat	204 (57.96)	148 (42.05)	352 (7.34)
	Zefta	136 (48.23)	146 (51.77)	282 (5.88)
	Samanoud	124 (53.91)	106 (46.09)	230 (4.80)
	El Santa	98 (34.51)	186 (65.49)	284 (5.92)
	Kotour	77 (39.09)	120 (60.91)	197 (4.11)
	Basyoon	103 (47.25)	115 (57.75)	218 (4.55)
Stage†	I	94 (62.67)	56 (37.33)	150 (4.39)
	II	695 (60.28)	458 (39.72)	1153 (33.73)
	III	935 (59.55)	635 (40.45)	1570 (45.93)
	1V	290 (53.21)	255 (46.79)	545 (15.95)
Basis of Diagnosis#	Histology	2060 (62.18)	1253 (37.82)	3313 (77.14)
	FNAC	494 (66.67)	247 (33.33)	741 (17.25)
	Others	184 (76.35)	57 (23.65)	241 (5.61)

*1.5% of cases had missing residence information.
†28.7% of cases had missing or unknown AJCC stage information.
#10.4% of cases had missing information on basis of diagnosis.

Table 3.2. Breast cancer incidence rates by year for the entire region, and age-standardized rates for urban, rural and urban-rural incidence rate ratio in Gharbiah, Egypt, 1999-2006.

Year	No Cases	Overall Incidence per 100,000#	Age-Standardized Urban Incidence	Age-Standardized Rural Incidence	Urban-Rural IRR (95% CI)*
1999	560	31.75	65.81	14.22	4.63 (3.20, 6.69)
2000	541	30.22	62.57	13.90	4.50 (3.09, 6.57)
2001	577	31.81	61.58	16.76	3.67 (2.55, 5.29)
2002	621	33.67	68.97	15.62	4.42 (3.09, 6.31)
2003	613	32.93	53.42	20.34	2.63 (1.84, 3.75)
2004	665	35.11	59.87	20.13	2.97 (2.11, 4.20)
2005	617	31.81	55.81	19.71	2.83 (1.97, 4.06)
2006	681	34.49	59.21	21.87	2.71 (1.91, 3.83)
Overall	**4794**	**32.15**	**60.90**	**17.82**	**3.73 (3.30, 4.22)†**

*IRR = Incidence Ratio. CI = Confidence Interval. †Adjusted for age, stage and year of diagnosis. #*P* for trend = 0.02

Table 3.3. Comparison of breast cancer incidence rates* and incidence rate ratios between districts in Gharbiah, Egypt from 1999-2006.

Years	1999		2000		2001		2002		2003		2004		2005		2006	
Districts	**Inc†**	**IRR† (95% CI)**	**Inc**	**IRR (95% CI)**	**Inc**	**IRR (95% CI)**	**Inc**	**IRR (95% CI)**	**Inc**	**IRR (95% CI)**	**Inc**	**IRR (95% CI)**	**Inc**	**IRR (95% CI)**	**Inc**	**IRR (95% CI)**
Tanta	49.66	1.95 (1.32, 2.90)	42.34	1.48 (1.01, 2.15)	50.31	2.48 (1.61, 3.81)	51.99	2.22 (1.49, 3.30)	48.09	1.88 (1.26, 2.80)	50.10	2.06 (1.40, 3.04)	46.16	2.31 (1.50, 3.56)	46.69	2.11 (1.40, 3.17)
El Mehalla	34.19	1.35 (0.90, 2.01)	30.35	1.06 (0.72, 1.56)	35.15	1.74 (1.13, 2.70)	51.22	1.75 (1.17, 2.62)	36.12	1.41 (0.94, 2.12)	46.61	1.92 (1.30, 2.82)	40.94	2.05 (1.33, 3.16)	43.30	1.95 (1.30, 2.93)
Kafr El-Zayat	39.91	1.10 (0.69, 1.76)	32.88	0.98 (0.63, 1.53)	23.54	1.16 (0.70, 1.93)	35.13	1.50 (0.95, 2.36)	23.68	0.93 (0.57, 1.50)	19.93	0.82 (0.50, 1.34)	17.21	0.86 (0.50, 1.48)	25.93	1.17 (0.72, 1.89)
Zefta	29.41	0.81 (0.50, 1.32)	21.99	0.66 (0.41, 1.06)	19.01	0.94 (0.56, 1.58)	18.17	0.78 (0.47, 1.28)	17.36	0.68 (0.41, 1.12)	16.55	0.68 (0.42, 1.12)	16.15	0.81 (0.47, 1.38)	16.36	0.74 (0.44, 1.23)
Samanoud	15.20	0.60 (0.34, 1.06)	22.44	0.78 (0.51, 1.29)	17.67	0.87 (0.49, 1.54)	13.74	0.59 (0.33, 1.06)	20.59	0.80 (0.48, 1.36)	29.97	1.23 (0.77, 1.98)	17.34	0.87 (0.49, 1.54)	29.10	1.31 (0.80, 2.14)
El Santa	21.04	0.83 (0.50, 1.36)	23.16	0.81 (1.29, 0.51)	28.23	1.39 (0.84, 2.29)	20.68	0.89 (0.54, 1.46)	15.74	0.62 (0.36, 1.05)	19.46	0.80 (0.49, 1.31)	19.59	0.98 (0.58, 1.67)	20.99	0.95 (0.57, 1.57)
Kotour	21.59	0.85 (0.50, 1.43)	28.11	0.98 (0.61, 1.58)	17.97	0.88 (0.50, 1.57)	14.71	0.63 (0.35, 1.12)	12.99	0.51 (0.28, 0.92)	12.05	0.50 (0.27, 0.90)	22.04	1.10 (0.64, 1.90)	16.60	0.75 (0.43, 1.31)
Basyoon‡	25.42	1.00	28.64	1.00	20.30	1.00	23.42	1.00	25.60	1.00	24.30	1.00	19.97	1.00	22.17	1.00

*All incidences are per 100,000 women. †Inc = Incidence, IR = Incidence ratio
‡Basyoon is the reference district

Figure 3.1. Map of Nile Delta Region showing location of eight districts of Gharbiah with the respective overall incidence rates of breast cancer in each district.

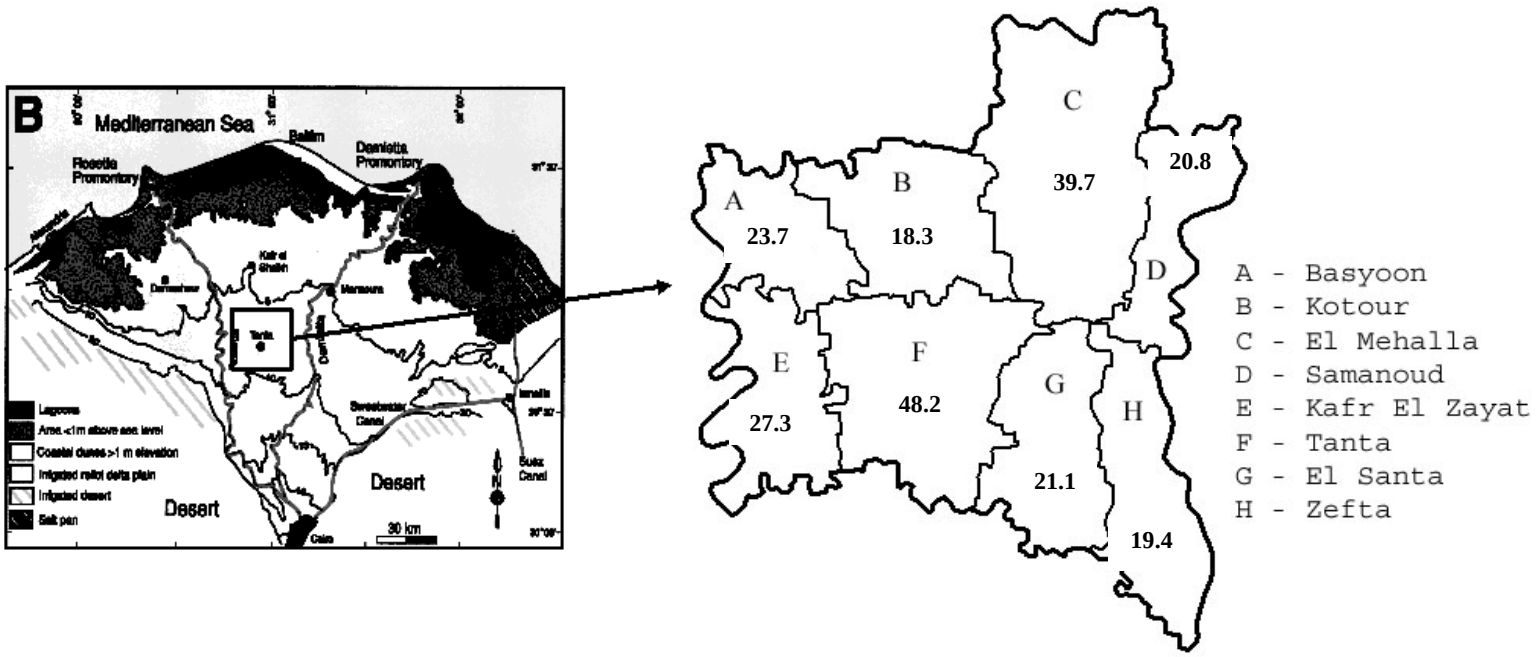


Figure 3.2. Overall age-specific incidence of breast cancer in Gharbiah, Egypt from 1999-2006. There are 12 categories of age: 0-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74, >75, each represented by a point on the graph for each year in that sequence.

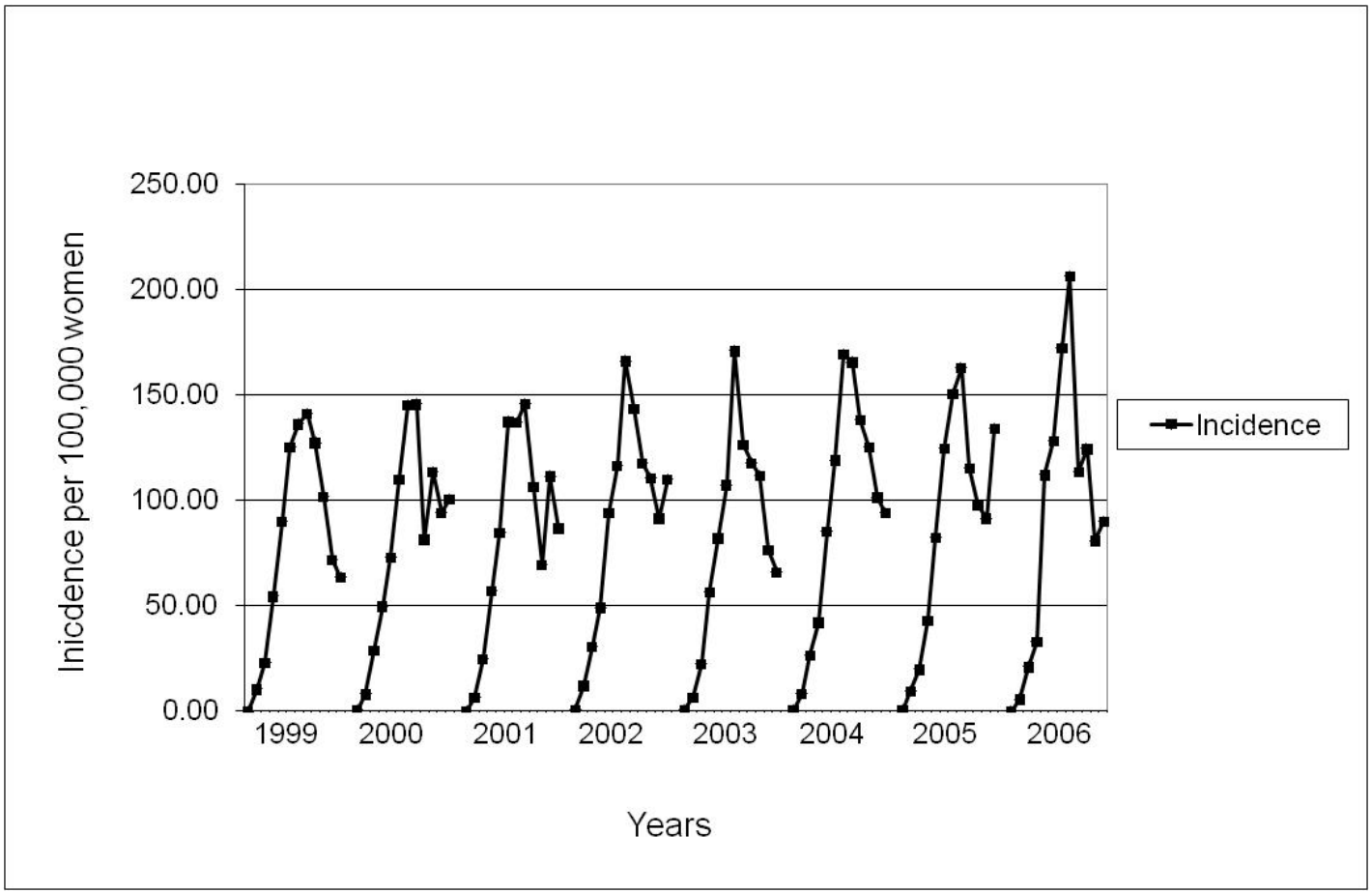
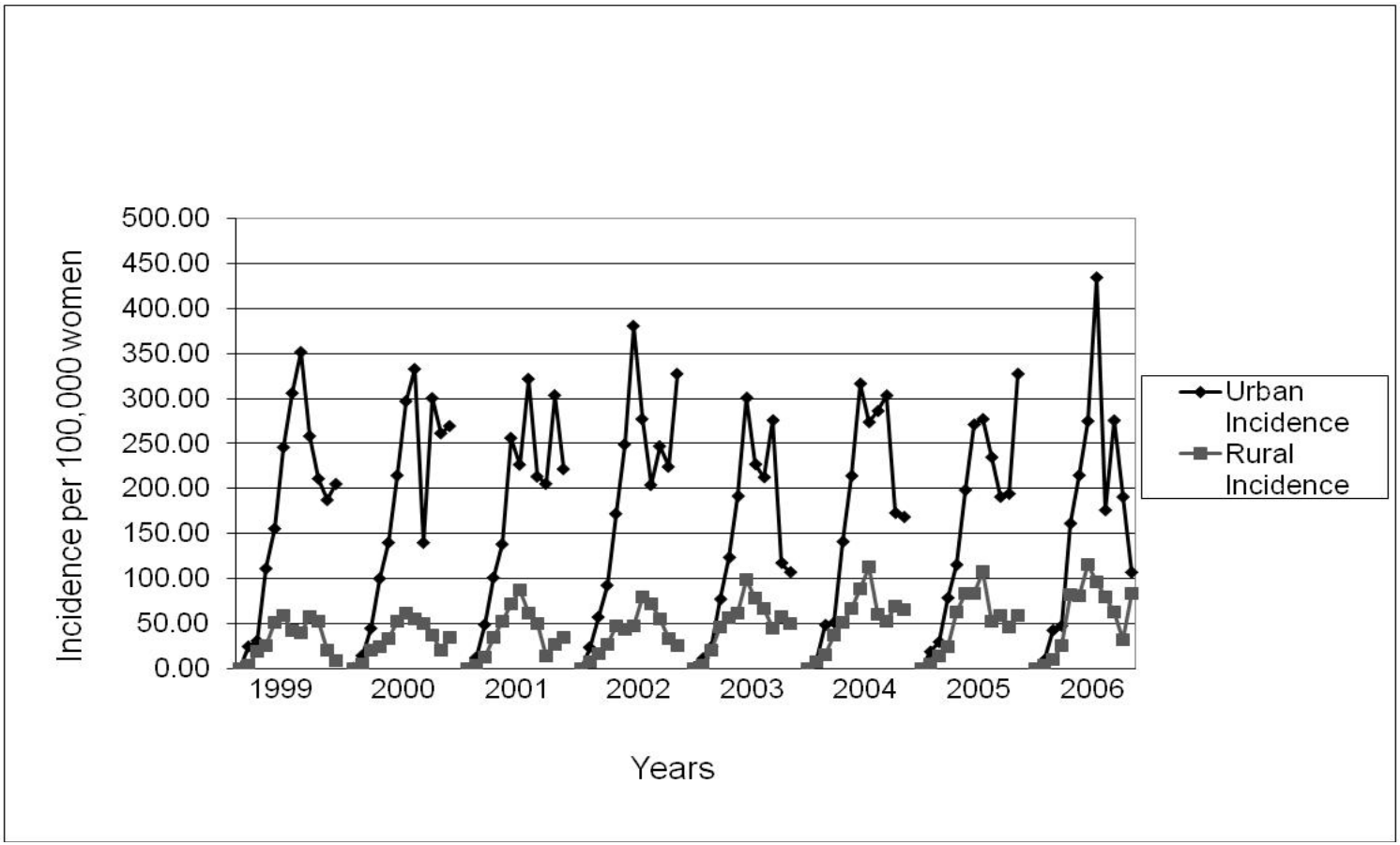


Figure 3.3. Urban-rural age-specific incidence of breast cancer in Gharbiah, Egypt from 1999-2006. There are 12 categories of age: 0-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74, >75, each represented by a point on the graph for each year in that sequence.



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CHAPTER IV

URBAN-RURAL DIFFERENCES IN BREAST CANCER INCIDENCE BY HORMONE RECEPTOR STATUS ACROSS 6 YEARS IN EGYPT: A LOCAL PHENOMENON IN A GLOBAL PATTERN

INTRODUCTION

Breast cancer incidence differs across various populations with higher incidence rates seen among populations living in industrialized or developed countries [1]. However, breast cancer is not a homogeneous disease and there are various subtypes of this malignancy. One of the most important ways of dividing breast cancer into subtypes is by the use of hormone receptor status (HRS) [2]. The need to develop these subtypes of breast cancer arose due to the differential response of breast tumors to different modes of therapy based on the presence or absence of receptors [3]. Presence of estrogen receptors (ERs) and progesterone receptors (PRs) or ER+/PR+ or hormone receptor positive (HR+) breast cancer, implies the best response of a tumor to anti-estrogen therapy whereas absence of these receptors or ER-/PR- or hormone receptor negative (HR-) breast cancer, implies poor response to anti-estrogen therapy. These differences are more pronounced if we take into account only the ER status of tumors [3]. The natural history of disease between ER+ and ER- tumors varies with better prognosis overall seen for ER+ patients. Patterns of relapse also differ with the site of recurrence more common in viscera and soft tissue for ER- cancers and in bone for ER+ cancers. The probability of recurrence is

highest within the first 5 years for ER- cancers while ER+ cancers tend to relapse later [4,5]. Epidemiological analysis of breast cancer by hormone receptor status also shows distinct patterns for ER+ and ER- cancers. Risk factor distribution differs among patients based on hormone receptor status with most reproductive factors that increase a woman's lifetime exposure to endogenous estrogens resulting in ER+ breast cancer [6,7]. Other risk factors such as genetic risks, radiation and smoking give rise to ER- breast cancers [6,8]. Overall, these differences clearly imply that ER+ and ER- cancers denote different subtypes of breast cancer with different risk factors, clinical pictures and outcomes [3].

Another very interesting characteristic difference of ER+ and ER- breast cancer is the close correlation of ER+ breast cancer incidence with populations in which breast cancer incidence is high. It is a known fact that in developed countries breast cancer incidence increases in later ages, mostly after menopause [9]. Studies involving the Surveillance Epidemiology and End Results (SEER) show ER+ cancers to be more frequent after menopause [10,11] and to be more common among Caucasians than other races [12,13, 14]. In addition, international studies have also clearly indicated that ER+ breast cancer is higher in developed countries where breast cancer incidence is the highest [15]. Thus, high incidence of ER+ breast cancer seems to be the hallmark of populations with high incidence of breast cancer. A very significant study to support this correlation comes from Li *et al* [16] who showed that most of the increase in breast cancer incidence in US has been due to an increase in ER+ breast cancer.

Thus, existing evidence suggests that ER+ breast cancer is high and increasing in the industrialized parts of the world and ER+ breast cancer is mainly due to risk factors that are estrogenic in nature. However, most of the reproductive and lifestyle factors that

increase a woman's lifetime exposure to estrogen explain only up to 50% of breast cancer risk [17,18]. This implies that there are other unknown estrogenic risk factors that increase a woman's risk of developing breast cancer, mostly later in life. Such estrogenic risk factors that are related to industrialization and development are chemicals known as xenoestrogens. These xenoestrogens include chemicals used in plastics such as bisphenol-A (BPA), phthalates and polyvinyl chloride (PVC), pesticides and insecticides like DDTs, polychlorinated biphenyls (PCBs), aldin, dieldrin, chlordane, lindane etc., parabens and placental extracts in cosmetics, aromatic amines, and industrial solvents like benzene and toluene, and products of air pollution such as polyaromatic hydrocarbons (PAHs) [19]. As is apparent xenoestrogens pervade almost all areas of modern life in developed parts of the world [19].

There is increasing evidence that xenoestrogens are related to breast cancer [19,20]. At the molecular level studies have shown that exposure to xenoestrogens preferably results in ER+ breast cancer [21-24]. However, studies in humans have been equivocal and the evidence for showing that xenoestrogens are a risk factor for breast cancer is limited. One of the most important reasons for this is the lack of comparison between populations that are differentially exposed to xenoestrogens since in the developed countries of the world the exposure to these xenoestrogens is almost ubiquitous [25]. Studies looking at the levels of some of these chemicals in the blood and urine of individuals in US have found that more than 90% of the US population has appreciable levels of these chemicals in their body [26-29]. However, populations differentially exposed to xenoestrogens are available in developing world due to differential levels of industrialization and development. One such set of populations that

have differential rates of development (and consequently differential exposure to xenoestrogens) is urban and rural populations in developing countries. There have been multiple studies from different parts of the world showing higher presence and exposure to xenoestrogens in urban areas [30-38]. Since urban populations are more exposed to xenoestrogens and it can be hypothesized that they should have higher incidence of breast cancer as well as ER+ breast cancer when compared to rural areas. We have recently published this set of hypotheses [39] and we have already shown in a recent study that incidence of breast cancer is indeed higher in urban areas when compared to rural areas in Egypt [40]. The purpose of this study was to investigate the urban-rural differences in breast cancer incidence by hormone receptor status to test the above hypothesis in the same population as the previous study.

METHODS

Study Population. The study population consisted of all women diagnosed with primary breast cancer with known ER or PR status from 2001 to 2006, a total period of 6 years, in the Gharbiah population-based cancer registry, Tanta, Egypt. ER and PR information was not routinely entered in the registry database especially for the years 2001 – 2004. Therefore, for all cases lacking this information, medical records were obtained from Tanta Cancer Center (TCC), Gharbiah Cancer Society (GCS) and any other centers for which medical records were available. Cases' registry number, age at diagnosis, address, address code, smoking status, occupation, basis of diagnosis, estrogen receptor status, progesterone receptor status, tumor grade, stage, morphology, medical record number and place of reference were abstracted from the routinely collected registry data. Use of

human subject data was approved by the University of Michigan Institutional Review Board and the Gharbiah Cancer Center Ethics Committee.

Gharbiah Population-Based Cancer Registry. The Gharbiah population-based cancer registry was founded in 1998 as a part of the Middle East Cancer Consortium (MECC) and is located in Tanta, the capital of Gharbiah province [41]. As an active registry, it collects cases from a number of sources in the province to determine cancer incidence. Most of the breast cancer cases came from Tanta Cancer Center (40-50%), Gharbiah Cancer Society (10-12%) and Tanta University Hospital (10-12%). The remaining cases came from pathology laboratories (10%), Mansoura Radiotherapy and Nuclear Medicine Department (3-4%), Insurance hospitals (4-5%), NCI, Cairo (2-3%) and mortality records (4-5%). In the years 1999-2002 registry staff visited the respective hospitals and centers to abstract data from medical records. After 2002, the registry has been copying and collecting the records from these centers, followed by abstraction of the data on a form that conforms to the International Agency for Research on Cancer (IARC) software CanReg4 permitting electronic entry in the CanReg4 database. Registry staff was trained in data extraction and entry methods, and are periodically monitored by site visits from the faculty of Emory School of Public Health, IARC, and the MECC registry Steering Committee members.

Most of the cases (95.8%) were diagnosed by pathological confirmation [42]. The World Health Organization (WHO) ICD-02 coding was used to determine the types of cancer in 1999 and 2000 after which ICD-03 coding was used. Cases were registered with SEER staging information from 1999-2002 and the American Joint Committee on

Cancer (AJCC) staging was begun in the registry only from 2003, although records for patients were retrieved and all previous SEER staging was converted to AJCC staging.

ER and PR Determination. ER and PR status was determined by immunohistochemistry (IHC) in all the centers providing cases to the registry. Paraffin sections of tissues are boiled in 10mM citrate buffer for 10-20 minutes followed by cooling at room temperature for 20 minutes. Monoclonal antibodies for ER and PR are then added to separate tissue sections and incubated for 30 minutes followed by visualization. The percentage of stained cells and strength of staining determines the score of positivity for ER and PR (1+, 2+ or 3+) with presence of stain in <1% cells or weak staining implying receptor negative status [43]. For our analyses we dichotomized the hormone receptor status into either positive or negative.

Gharbiah Province. Gharbiah province is an administrative region located 90 kilometers north of Cairo in the Nile Delta Region. It has eight districts each with a capital city with Tanta being the capital of Tanta district as well as of the entire province. Gharbiah has a population of more than 4 million people and 49% of them are women. Approximately, 30% of the population resides in urban areas and almost 47% of the female population is below the age of 20 according to the 2006 Central Agency for Public Mobilization and Statistics (CAPMAS) census. Most of the residents residing in rural areas are part of an agricultural economy but most people living in cities participate in industrial occupations with most of the industries located in the two of the largest districts of Tanta and El Mehalla.

Census Data. Census data for female population in Gharbiah was obtained from the 1996 and 2006 CAPMAS [44] census and constant growth of the population was assumed to project populations in the years in-between using linear regression model. The linear growth rates of eight districts were applied to the urban and rural populations within that district to determine urban and rural populations from 1999 through 2006. The census data consisted of 16 age categories at 5 year intervals. Six age categories were created from these by collapsing the age categories below 29 years followed by 10 year intervals. These population figures formed the denominators to calculate the overall, age-specific, district specific and urban-rural incidence rates for breast cancer in women.

Urban Rural Classification. The urban rural classification followed the CAPMAS coding of urban and rural areas [44]. Urban areas consisted of all the capital cities of the eight districts of the province while the villages surrounding the capital cities and villages in rest of the district were considered rural. Each case in the registry is assigned a residence code based on their residential address that follows the CAPMAS coding. This code was used to classify patients as urban or rural. Although there are a number of ways to define urban and rural populations, this code represented people living either in agricultural economy present predominantly in villages or an industrial economy present predominantly in cities.

Statistical Analyses. Descriptive statistics and incidence rate analyses were completed using SAS (Version 9; SAS Institute, Cary, NC). Yearly crude and age adjusted

incidence rates for breast cancer were calculated for Gharbiah province and the six age categories classified by urban and rural areas for the province and each age category. The six age categories were 0-29, 30-39, 40-49, 50-59, 60-69 and 70 or more. We stratified our analyses by hormone receptor status, specifically assessing separately, the rates of ER+, ER-, PR+, PR-, ER+/PR+, ER+/PR-, ER-/PR+ and ER-/PR- tumors. Women with ER or PR status that was unknown or could not be assessed were excluded from the analyses. To account for the excluded cases, we computed the proportion of women with a particular HRS (among those with known hormone status) by year of incidence, age group and urban-rural status. We then calculated urban-rural incidence rate ratios (IRRs) and 95% confidence intervals (CI). We also evaluated how the incidence of a given hormone receptor status changed over time.

We considered age, stage and year of diagnosis as potential confounders in our analysis. To assess overall differences in urban-rural incidence and trends in incidence rates we used negative binomial regression to calculate IRRs and associated 95% CIs along with *P*-values for trends over the period of six years.

RESULTS

This study examined a total of 3673 cases for a total of 6 years – 2001 to 2006. The baseline characteristics of the cases in this study have been described elsewhere [40]. ER status was known for 47.63% of cases and PR status was known for 37.19% of cases on an average (Table 4.1). The proportion and incidence of cases with unknown ER and PR receptor status was almost similar for all the years. Among all the cases on an average 32.82% were ER+, and 14.82% were ER-. Among all the cases, on an average 21.55%

were PR+ and 15.62% were PR-. The average incidence of ER+ breast cancer was the highest (10.92 per 100,000 women) followed by PR+ breast cancer (7.18 per 100,000 women), PR- breast cancer (5.18 per 100,000 women) and ER- breast cancer (4.93 per 100,000 women) (Table 4.1). Distribution of breast cancer cases by joint receptor status shows that on an average ER+/PR+ breast cancer had the highest incidence (6.44 per 100,000 women) followed by ER-/PR- breast cancer (3.74 per 100,000 women). The incidence of ER+/PR- breast cancer was low (1.44 per 100,000 women) followed by lowest incidence of ER-/PR+ breast cancer (0.73 per 100,000 women) (Table 4.2). The proportion (average - 62.89%) and incidence (average - 20.88 per 100,000 women) of cases with unknown ER/PR status remained almost constant throughout the years. We did not see any noticeable trends in the incidence of breast cancer by hormone receptor status as is clear from the p-values for trend.

Urban-rural distribution of HRS shows that ER+ incidence was the highest followed by PR+ positive incidence within both urban and rural areas (Table 4.3). ER- and PR- rates are quite similar within both urban and rural areas. On comparison of urban and rural incidences, ER+ incidence in urban areas is 2-4 times higher than ER+ incidence in rural areas (2001; IR = 3.58, 95% CI = 4.82, 2.65, 2006; IR = 2.45, 95% CI = 3.08, 1.94 and overall IR = 3.36, 95% CI = 4.84, 2.34) (Table 4.3). This is followed by PR+ incidence which is 2-4 times higher in urban areas than in rural areas (2001; IR = 3.57, 95% CI = 5.27, 2.42, 2006; IR = 2.77, 95% CI = 3.63, 2.11 and overall; IR = 2.29, 95% CI = 3.70, 1.70). ER- (2001; IR = 1.82, 95% CI = 2.93, 1.13, 2006; IR = 3.00, 95% CI = 4.40, 2.05 and overall; IR = 1.86, 95% CI = 2.38, 1.45) and PR- (2001; IR = 1.93, 95% CI = 2.87, 1.30, 2006; IR = 2.71, 95% CI = 4.02, 1.82 and overall; IR = 1.89, 95%

CI = 2.24, 1.60) (Table 4.3) breast cancer incidence is almost 2-3 times higher in urban than in rural areas.

Urban-rural distribution of joint HRS shows that ER+/PR+ breast cancer incidence in urban areas is the highest, being 2-4 times that in rural areas (2001; IR = 3.41, 95% CI = 5.08, 2.29, 2006; IR = 2.59, 95% CI = 3.46, 1.94 and overall; IR = 2.33, 95% CI = 3.23, 1.68) (Table 4.4). ER-/PR- breast cancer is also 1-3 times higher in urban areas than in rural areas (2001; IR = 1.47, 95% CI = 2.46, 0.87, 2006; IR = 2.89, 95% CI = 4.75, 1.76 and overall; IR = 1.72, 95% CI = 2.32, 1.28) (Table 4.4). ER+/PR- and ER-/PR+ cases were very few and therefore they were not included in further analysis in Table 4.4.

Age-specific distribution of breast cancer incidence by HRS shows higher incidence for all receptors in urban areas when compared to rural areas (Table 4.3) (Figure 4.1). Within urban areas ER+ incidence is the highest in all age-groups followed by PR+ incidence. ER- and PR- breast cancer incidence is almost similar. Within rural areas the incidence of all four receptor types is almost similar with slightly higher incidence for ER+ and PR+ in 2006. Comparison of urban-rural incidences shows that the incidence of all hormone receptor types is higher in urban areas than in rural areas for all age-groups with the incidence of ER+ breast cancer being the highest in all age-groups (Table 4.3). Age-specific distribution of breast cancer incidence by joint HRS shows that ER+/PR+ breast cancer incidence is highest in urban areas (Table 4.4) (Figure 4.2) in most age-groups. Within rural areas, ER+/PR+ and ER-/PR- breast cancer incidence is almost similar except in 2006.

DISCUSSION

This is the first study from a population-based cancer registry in a developing country to show higher ER+ breast cancer incidence in urban areas. This study further confirms the hypothesis that populations with higher incidence of breast cancer also demonstrate higher incidence of ER+ breast cancer. This pattern is visible both in comparison of HRS incidence and age-specific incidence classified by HRS. The reason for higher incidence of ER+ breast cancer in urban areas is multi-factorial. It is quite possible that women in urban areas have better nutrition and development which leads to early menarche. They might be more educated which results in higher age of marriage, lesser number of children and reduced breastfeeding [45]. All of these reproductive factors result in higher lifetime exposure of women to endogenous estrogens and thus can increase ER+ breast cancer.

However, we have already shown in our recent study that breast cancer incidence is 3-4 times higher in urban areas of Egypt and this cannot be explained by known reproductive risk factors [40], a fact that has also been seen in other populations [17,18]. Thus, other risk factors such as exposure to xenoestrogens might play a very important role in increasing ER+ breast cancer in cities. Women in urban areas are prone to using more plastics and electrical appliances, household insecticides, detergents, cosmetics etc. They are also exposed more to air pollution – both vehicular and industrial, which is a source of PAHs [46]. Research shows that there is extensive pollution of the Nile River which is the primary water source in Egypt as well as Nile Delta soil [47-49]. In addition food products which arrive in cities, both vegetarian and non-vegetarian, are more

processed or have higher levels of preservatives such as pesticides which further increase the exposure of urban populations to xenoestrogens [50]. Our group showed in a previous study that urban women also have higher levels of 7,8-dihydro-8-oxo-2'-deosyguanine (8-oxo-dG) which indicates higher DNA damage and consequently implies higher exposure to carcinogens [51]. Since xenoestrogens have estrogenic effects and have been shown to be related to ER+ breast cancer [21-24], all the above exposures lead to higher incidence of breast cancer and preferentially ER+ breast cancer in urban areas. That apart, within rural areas the incidence of breast cancer is almost similar for all HRS and this pattern is more pronounced when looking at age-specific incidence rates. This demonstrates that exposure to estrogenic and non-estrogenic risk factors are quite similar in rural areas while in urban areas exposure to estrogenic factors is higher.

ER status of breast cancer is also related to the period in women's life when they are exposed to various risk factors. This link of breast cancer to exposures during particular times in women's life arises from studies into breast development and stem cell research. There are three critical periods in the development of mammary glands: the intrauterine period especially just before birth, the peripubertal period and the period of pregnancy and lactation [52]. Research into mammary stem cells, which are now considered to be the origin of breast cancer [53-57], tells us that during the intrauterine period all stem cells which are the progenitor stem cells are ER- [58,59]. Post-natally these ER- stem cells differentiate into ER+ cells which later form mammary glands during puberty under the influence of estrogen [58]. Although it is known that genetic and non-estrogenic factors cause ER- breast cancer [6] it is quite possible that early life exposure during the intrauterine period or around birth affect the progenitor stem cells

which at that time are predominantly ER- which would then lead to ER- breast cancer. However, progenitor stem cells are quite hardy and resistant to mutations [60] and as such in populations exposed to estrogenic risk factors early in life – endogenous or exogenous – ER- breast cancer would be higher than in unexposed populations but still lower than ER+ breast cancer within the same exposed populations. ER+ breast cancer must be higher in exposed populations because ER+ stem cells are more numerous later in life and as age progresses these stem cells lose some of their resistance to mutations. This speculation arises from the model of about breast cancer development from stem cells based on ER status [60]. This also explains the higher incidence of breast cancer later in life after menopause in exposed populations.

Our observations closely matched the predictions that can be derived from the stem cell model and it was seen that ER- breast cancer is higher in urban areas. This shows that women in urban areas must have been exposed to etiological agents of breast cancer in the intrauterine period or early in life. Exposure to xenoestrogens in early life is quite plausible in the light of the evidence which shows excretion of xenoestrogens in human milk in Egypt [61] and across the world [62-64]. Thus, in urban areas women are exposed to higher levels of xenoestrogens from fetal stage which increases the likelihood of ER- breast cancer later in life due to mutations in progenitor stem cells. However, progenitor stem cells are resistant to mutations and less numerous [60] due to which ER- breast cancer incidence is not much higher in urban areas than in rural areas. Later in life progenitor stem cells differentiate into intermediate stem cells that are ER+. These intermediate stem cells are more numerous and less resistant to mutations [60]. Thus, higher lifetime exposure of women to xenoestrogens in urban areas leads to higher ER+

breast cancer incidence, more so later in life after menopause when the ER+ stem cells accumulate maximum number of mutations according to the multi-hit theory of carcinogenesis.

PR expression is under the control of ER expression [65, 66] and as such the pattern of PR expression closely follows ER expression. However, not all ER+ tumors express PR and thus PR+ incidence is lower than ER+ incidence. Also, since some ER+ tumors are PR-, PR- incidence must be slightly higher or similar to ER- incidence, a pattern seen clearly in Gharbiah. Breast cancer incidence by joint HRS can also be explained due to above reason since ER+/PR+ incidence is the highest followed by ER-/PR- incidence. ER+/PR- incidence is next since some ER+ tumors don't express PR. ER-/PR+ incidence is the lowest since in the absence of ER expression PR expression is very unlikely. Age-specific incidence by joint HRS was limited to ER+/PR+ and ER-/PR- breast cancer since the number of cases in other two joint hormone receptor categories was too low for some age-groups. ER+/PR+ breast cancer has the highest incidence for most age-groups within urban areas and also when compared to rural areas. Within rural areas incidence of ER+/PR+ and ER-/PR- breast cancer is similar for most age-groups in all years which again clearly shows that estrogenic and non-estrogenic exposures are almost similar in rural areas.

One of the main limitations of this study is the absence of HRS information for all the cases for the six year period. It can be seen that the incidence of cases with unknown HRS has remained almost similar across the years with decrease in 2006 since we had information on more number of cases for this year. Still this did not affect the urban-rural differences of breast cancer by HRS in 2006 substantially. We also compared cases

with known HRS with cases with unknown HRS and cases from entire Gharbiah based on important baseline factors like urban-rural distribution, age and AJCC stage and found the three categories of cases to be similar to each other in these distributions. Also, a number of cases with missing HRS information were diagnosed by FNAC and many of these cases were having metastatic disease (Stage 4) which is more likely to be ER- and such cases are more likely to be rural. Detection of HRS of such cases would increase ER- incidence in rural areas which will not affect ER+ incidence in urban areas. Thus, it is unlikely that absence of HRS information affected our findings in this study.

Another confounder of our findings could be the difference of HRS determination among the various pathology laboratories in Gharbiah. However, HRS determination was started routinely for most cases in 2001 and as such the method for HRS determination was quite standardized across the world by then. We also obtained details of the procedure from various laboratories and determined that HRS determination was similar for all sites since 2001. Thus, differences in procedures for determining HRS are also unlikely to affect our findings [16]. In addition, the differences, even if they exist, are more with regards to classifying the degree of positivity and not regarding classifying tumors as positive and negative. Since we have based our analysis in this paper on classifying tumors into positive or negative the effects of any subjective difference between laboratories is quite minimal.

Overall we were able to show from a population-based cancer registry that urban women have a higher incidence of ER+ breast cancer than rural women. This is the same population which also had higher incidence of breast cancer as such. This finding is consistent with findings from other populations around the world which have shown that

higher incidence of ER+ breast cancer is closely associated with populations with higher incidence of breast cancer. Apart from known and well-studied estrogenic risk factors of breast cancer xenoestrogens might be a very significant cause of high incidence of breast cancer and more specifically ER+ breast cancer in developed countries and urban areas of developing countries. The pattern of distribution of HRS in urban and rural areas also point towards probable timing of exposure to xenoestrogens.

Future studies must investigate individual level exposures of women to xenoestrogens which need to be correlated to HRS of breast cancer to confirm this phenomenon. More studies are also needed to look closely at the molecular mechanisms by which xenoestrogens lead to ER+ breast cancer. We also need to look closely at the role of mammary stem cells since they might hold important clues regarding critical periods of exposure and better ways of prevention and treatment of breast cancer.

Table 4.1. Distribution of number, percentage and overall incidence* of breast cancer by hormone receptor status in Gharbiah, 2001-2006.

	ER+			ER-			ER Unknown			PR+			PR-			PR Unknown		
Year	**No.**	**%**	***Inc****	**No.**	**%**	***Inc****	**No.**	**%**	***Inc****	**No.**	**%**	***Inc****	**No.**	**%**	***Inc***	**No.**	**%**	***Inc****
2001	188	32.58	*10.37*	68	11.79	*3.75*	321	55.63	*17.70*	111	19.24	*6.12*	98	16.98	*5.40*	368	63.78	*20.29*
2002	172	27.70	*9.33*	92	14.81	*4.99*	357	57.49	*19.36*	98	15.78	*5.31*	99	15.94	*5.37*	424	68.28	*22.99*
2003	187	31.17	*10.19*	100	16.67	*5.45*	313	52.17	*17.06*	130	21.67	*7.09*	109	18.17	*5.94*	361	60.17	*19.68*
2004	192	29.45	*10.26*	91	13.96	*4.86*	369	56.60	*19.71*	120	18.41	*6.41*	86	13.19	*4.59*	446	68.41	*23.83*
2005	180	30.72	*9.77*	86	14.68	*4.67*	320	54.61	*17.37*	121	20.65	*6.57*	81	13.82	*4.40*	384	65.53	*20.84*
2006	293	45.29	*15.62*	110	17.00	*5.86*	244	37.71	*13.01*	217	33.54	*11.57*	101	15.61	*5.38*	328	50.70	*17.49*
Overall	1212	32.82	*10.92*	547	14.82	*4.93*	1924	52.37	*17.37*	797	21.55	*7.18*	574	15.62	*5.18*	2311	62.81	*20.85*
***p* for trend†**			*0.93*			*0.91*			*0.95*			*0.98*			*0.99*			*0.91*

*All incidences are per 100,000 women.
†Adjusted for stage and year of diagnosis.

Table 4.2. Distribution of number, percentage and overall incidence* of breast cancer by joint hormone receptor status in Gharbiah, 2001-2006.

	ER+/PR+			ER+/PR-			ER-/PR+			ER-/PR-			ER/PR Unknown		
Year	**No.**	**%**	***Incidence****	**No.**	**%**	***Incidence****	**No.**	**%**	***Incidence****	**No.**	**%**	***Incidence****	**No.**	**%**	***Incidence****
2001	105	18.20	*5.79*	39	6.76	*2.15*	6	1.04	*0.33*	58	10.52	*3.20*	369	63.95	*20.34*
2002	86	13.85	*4.66*	27	4.35	*1.46*	12	1.93	*0.65*	72	11.59	*3.90*	424	68.28	*22.99*
2003	118	19.67	*6.43*	27	4.50	*1.47*	12	2.00	*0.65*	82	13.67	*4.47*	361	60.17	*19.68*
2004	111	17.03	*5.93*	14	2.15	*0.75*	9	1.38	*0.48*	72	11.04	*3.85*	446	68.41	*23.83*
2005	105	17.92	*5.70*	16	2.73	*0.87*	16	2.73	*0.87*	65	11.09	*3.53*	384	65.53	*20.84*
2006	190	29.37	*10.13*	36	5.56	*1.92*	26	4.02	*1.39*	65	10.05	*3.47*	330	51.01	*17.59*
Overall	715	19.34	*6.44*	159	4.34	*1.44*	81	2.18	*0.73*	414	11.25	*3.74*	2314	62.89	*20.88*
***p* for trend†**			*0.98*			*0.87*			*0.98*			*0.97*			*0.91*

*All incidences are per 100,000 women.
†Adjusted for stage and year of diagnosis.

Table 4.3. Urban-rural incidence rates* and incidence ratios of breast cancer by ER/PR status, by age-groups and overall in Gharbiah, 2001-2006.

ER+	Urban-Rural Incidence and Incidence Ratios																				
	Age-Groups (Years)																		Overall		
	0-29			30-39			40-49			50-59			60-69			70+					
Years	**Urban**	**Rural**	***Urb-Rur IRR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IRR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IRR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IRR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IRR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IRR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IRR (95% CI)***
2001	0.59	0.12	*4.80 (0.44, 52.99)*	22.4	5.85	*3.80 (1.77, 8.17)*	59.17	17.76	*3.33 (1.96, 5.65)*	101.55	33.33	*3.05 (1.83, 5.08)*	60.75	15.47	*3.93 (1.67, 9.26)*	72.10	7.51	*9.60 (2.04, 45.19)*	19.88	5.56	*3.58 (2.65, 4.82)*
2002	0.58	0.24	*2.40 (0.34, 17.05)*	21.91	5.76	*3.80 (1.77, 8.17)*	50.99	10.83	*4.71 (2.49, 8.90)*	97.36	21.88	*4.45 (2.48, 8.00)*	63.81	24.75	*2.58 (1.24, 5.36)*	44.37	14.80	*3.00 (0.81, 11.17)*	18.24	4.77	*3.83 (2.79, 5.25)*
2003	1.15	0.24	*4.80 (0.88, 26.23)*	16.99	7.36	*2.31 (1.10, 4.85)*	54.40	17.19	*3.16 (1.86, 5.39)*	108.6	24.19	*4.49 (2.58, 7.80)*	74.46	16.84	*4.42 (2.00, 9.78)*	34.88	3.64	*9.60 (1.07, 85.85)*	18.83	5.19	*3.63 (2.68, 4.91)*
2004	0.00	0.23	*0.00*	12.23	8.34	*1.47 (0.67, 3.19)*	66.05	16.07	*4.11 (2.44, 6.94)*	114.24	26.39	*4.33 (2.56, 7.33)*	76.95	11.02	*6.98 (2.80, 17.39)*	25.68	10.71	*2.40 (0.48, 11.89)*	19.00	5.21	*3.65 (2.71, 4.91)*
2005	0.88	0.12	*7.21 (0.75, 69.29)*	23.05	1.73	*13.33 (3.96, 44.87)*	42.24	19.15	*2.21 (1.28, 3.81)*	110.49	27.34	*4.04 (2.37, 6.88)*	83.73	9.52	*8.80 (3.32, 23.33)*	53.23	25.89	*2.06 (0.69, 6.12)*	19.46	4.87	*4.00 (2.93, 5.46)*
2006	0.86	0.24	*3.60 (0.60, 21.57)*	22.64	10.75	*2.11 (1.12, 3.94)*	87.26	40.08	*2.18 (1.49, 3.17)*	139.54	47.00	*2.97 (1.94, 4.54)*	74.41	31.78	*2.34 (1.22, 4.50)*	61.00	25.43	*2.40 (0.84, 6.84)*	25.69	10.49	*2.45 (1.94 3.08)*
Overall IRR† and *p* for trend†																			***0.99***	***0.92***	***3.36 (2.34 4.84)***

Table 4.3 (continued)

ER-	Urban-Rural Incidence and Incidence Ratios																			
	Age-Groups (Years)																		Overall	
	0-29			30-39			40-49			50-59			60-69			70+				
Years	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***
2001	0.59	0.12	*4.80 (0.44, 52.99)*	5.85	3.51	*1.67 (0.51, 5.46)*	19.23	15.23	*1.26 (0.62, 2.58)*	21.28	11.11	*1.92 (0.72, 5.13)*	16.20	1.93	*8.38 (0.94, 74.97)*	9.01	3.76	*2.40 (0.15, 38.36)*	5.38	2.95	*1.82 (1.13, 2.93)*
2002	0.58	0.36	*1.60 (0.27, 9.58)*	12.68	7.49	*1.69 (0.76, 3.78)*	20.39	7.50	*2.72 (1.18, 6.29)*	42.10	16.41	*2.57 (1.21, 5.42)*	7.98	11.42	*0.70 (0.14, 3.46)*	26.62	3.70	*7.20 (0.75, 69.19)*	7.68	3.62	*2.12 (1.41, 3.20)*
2003	0.57	0.12	*4.80 (0.44, 52.99)*	10.20	7.36	*1.38 (0.59, 3.24)*	20.04	14.73	*1.36 (0.68, 2.73)*	31.03	21.50	*1.44 (0.68, 3.05)*	27.43	13.09	*2.09 (0.73, 5.97)*	8.72	0.00	-	6.89	4.46	*1.55 (1.04, 2.29)*
2004	0.28	0.35	*0.80 (0.08, 7.70)*	8.90	4.45	*2.00 (0.75, 5.33)*	25.30	13.66	*1.85 (0.95, 3.59)*	22.85	9.24	*2.47 (0.92, 6.64)*	34.63	12.86	*2.69 (1.00, 7.23)*	0.00	10.71	-	6.94	3.55	*1.95 (1.29, 2.95)*
2005	0.29	0.12	*2.40 (0.15, 38.41)*	6.92	2.88	*2.40 (0.73, 7.86)*	20.39	10.83	*1.88 (0.89, 4.01)*	21.05	16.41	*1.28 (0.52, 3.14)*	39.87	13.32	*2.99 (1.14, 7.86)*	53.23	11.10	*4.80 (1.20, 19.19)*	7.24	3.38	*2.14 (1.40, 3.27)*
2006	0.57	0.00	-	4.53	3.40	*1.33 (0.38, 4.73)*	32.90	15.54	*2.12 (1.15, 3.89)*	59.43	13.43	*4.43 (2.11, 9.30)*	47.00	13.09	*3.59 (1.41, 9.12)*	26.14	3.63	*7.20 (0.75, 69.19)*	10.49	3.50	*3.00 (2.05, 4.40)*
Overall IRR† and *p* for trend†																			***0.83***	***0.86***	***1.86 (1.45, 2.38)***

Table 4.3 (continued)

PR+	Urban-Rural Incidence and Incidence Ratios																			
	Age-Groups (Years)																		Overall	
	0-29			30-39			40-49			50-59			60-69			70+				
Years	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***
2001	0.00	0.12	*0.00*	16.39	3.51	*4.67 (1.79, 12.14)*	28.11	11.00	*2.56 (1.26, 5.18)*	64.14	19.44	*3.30 (1.71, 6.38)*	40.50	5.80	*6.98 (1.92, 25.37)*	45.06	7.51	*6.00 (1.16, 30.91)*	11.73	3.28	*3.57 (2.42, 5.27)*
2002	0.29	0.24	*1.20 (0.11, 13.25)*	11.53	2.31	*5.00 (1.57, 15.94)*	30.59	8.33	*3.67 (1.73, 7.80)*	47.37	16.41	*2.89 (1.39, 5.99)*	23.93	17.13	*1.40 (0.50, 3.92)*	17.75	11.10	*1.60 (0.27, 9.57)*	9.28	3.29	*2.82 (1.89, 4.22)*
2003	0.86	0.12	*7.21 (0.75, 69.29)*	12.46	6.80	*1.83 (0.81, 4.16)*	38.65	13.10	*2.95 (1.59, 5.48)*	64.64	18.81	*3.44 (1.79, 6.61)*	43.11	13.09	*3.29 (1.28, 8.49)*	17.44	3.64	*4.80 (0.44, 52.91)*	12.09	4.13	*2.93 (2.06, 4.16)*
2004	0.00	0.23	-	8.90	5.00	*1.78 (0.69, 4.61)*	44.97	10.45	*4.31 (2.26, 8.20)*	58.39	19.79	*2.95 (1.54, 5.65)*	38.47	7.35	*5.24 (1.64, 16.70)*	8.56	10.71	*0.80 (0.08, 7.69)*	11.16	3.63	*3.07 (2.13, 4.44)*
2005	0.58	0.12	*4.80 (0.44, 52.99)*	13.83	0.58	*24.00 (3.12, 184.59)*	24.76	14.16	*1.75 (0.89, 3.43)*	60.51	16.41	*3.69 (1.84, 7.41)*	63.79	11.42	*5.59 (2.19, 14.28)*	62.11	25.89	*2.40 (0.84, 6.84)*	12.38	3.63	*3.41 (2.36, 4.94)*
2006	0.86	0.24	*3.60 (0.60, 21.57)*	13.59	7.92	*1.71 (0.79, 3.71)*	68.66	26.99	*2.54 (1.63, 3.96)*	116.28	30.89	*3.76 (2.28, 6.22)*	54.83	24.30	*2.26 (1.06, 4.80)*	52.29	14.53	*3.60 (1.02, 12.75)*	20.05	7.24	*2.77 (2.11, 3.63)*
Overall IRR† and *p* for trend†																			***0.97***	***0.99***	***2.29 (1.70, 3.70)***

Table 4.3 (continued)

PR-	Urban-Rural Incidence and Incidence Ratios																				
	Age-Groups (Years)																		Overall		
	0-29			30-39			40-49			50-59			60-69			70+					
Years	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***
2001	0.89	0.12	*7.21 (0.75, 69.29)*	5.85	5.85	*1.00 (0.34, 2.93)*	28.11	18.61	*1.51 (0.82, 2.79)*	32.07	15.28	*2.10 (0.93, 4.76)*	32.40	7.73	*4.19 (1.26, 13.91)*	18.02	3.76	*4.80 (0.44, 52.92)*	7.98	4.13	*1.93 (1.30, 2.87)*
2002	0.58	0.36	*1.60 (0.27, 9.58)*	11.53	8.07	*1.43 (0.63, 3.22)*	17.48	7.50	*2.33 (0.98, 5.53)*	52.63	17.78	*2.96 (1.47, 5.95)*	19.94	13.33	*1.50 (0.47, 4.71)*	35.50	0.00	-	8.48	3.78	*2.24 (1.51, 3.33)*
2003	0.57	0.24	*2.40 (0.34, 17.05)*	9.06	7.36	*1.23 (0.51, 2.97)*	20.04	13.91	*1.44 (0.71, 2.92)*	54.30	21.50	*2.53 (1.32, 4.84)*	31.35	13.09	*2.39 (0.87, 6.60)*	8.72	0.00	-	8.27	4.46	*1.85 (1.27, 2.70)*
2004	0.28	0.35	*0.80 (0.08, 7.70)*	6.67	5.00	*1.33 (0.47, 3.75)*	25.30	15.27	*1.66 (0.87, 3.16)*	27.92	3.96	*7.06 (1.97, 25.29)*	26.93	11.02	*2.44 (0.82, 7.27)*	0.00	7.14	*0.00*	6.64	3.32	*2.00 (1.31, 3.05)*
2005	0.29	0.12	*2.40 (0.15, 38.41)*	5.76	2.88	*2.00 (0.58, 6.91)*	13.11	11.66	*1.12 (0.49, 2.60)*	36.83	15.04	*2.45 (1.11, 5.39)*	35.88	9.52	*3.77 (1.26, 11.25)*	35.49	11.10	*3.20 (0.72, 14.29)*	6.76	3.22	*2.10 (1.36, 3.25)*
2006	0.00	0.00	-	9.06	3.40	*2.67 (0.93, 7.69)*	30.04	16.36	*1.84 (1.00, 3.39)*	41.34	13.43	*3.08 (1.40, 6.78)*	47.00	9.35	*5.03 (1.77, 14.27)*	17.43	3.63	*4.80 (0.44, 52.91)*	9.24	3.41	*2.71 (1.82, 4.02)*
Overall IRR† and *p* for trend†																			***0.90***	***0.80***	***1.89 (1.60, 2.24)***

*All incidences are per 100,000 women.

†Adjusted for age, stage and year of diagnosis.

Table 4.4. Urban-rural incidence rates* and incidence ratios of breast cancer by joint ER/PR status, by age-groups and overall in Gharbiah, 2001-2006[#].

ER+/PR+	Urban-Rural Incidence and Incidence Ratios																				
	Age-Groups (Years)																		Overall		
	0-29			30-39			40-49			50-59			60-69			70+					
Years	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***	**Urban**	**Rural**	***Urb-Rur IR (95% CI)***
2001	0.00	0.12	*0.00*	15.22	3.51	*4.33 (1.65, 11.40)*	23.67	11.00	*2.15 (1.04, 4.47)*	64.14	18.05	*3.55 (1.81, 6.98)*	36.45	5.80	*6.28 (1.70, 23,21)*	45.06	7.51	*6.00 (1.16, 30.91)*	10.91	3.20	*3.41 (2.29, 5.08)*
2002	0.00	0.24	*0.00*	10.38	1.73	*6.00 (1.62, 22.16)*	27.68	5.83	*4.75 (2.00, 11.29)*	39.47	15.04	*2.62 (1.21, 5.71)*	23.93	17.13	*1.40 (0.50, 3.92)*	17.75	11.10	*1.60 (0.27, 9.57)*	8.16	2.88	*2.84 (1.84, 4.36)*
2003	0.86	0.12	*7.21 (0.75, 69.29)*	9.06	6.80	*1.33 (0.55, 3.26)*	37.22	11.46	*3.25 (1.70, 6.22)*	62.06	14.78	*4.20 (2.06, 8.57)*	35.27	13.09	*2.69 (1.00, 7.23)*	17.44	3.64	*4.80 (0.44, 52.91)*	11.02	3.73	*2.96 (2.04, 4.28)*
2004	0.00	0.23	*0.00*	7.79	5.00	*1.56 (0.58, 4.18)*	42.16	10.45	*4.04 (2.11, 7.74)*	58.39	15.83	*3.69 (1.84, 7.41)*	34.63	5.51	*6.28 (1.70, 23.22)*	8.56	7.14	*1.20 (0.11, 13.23)*	10.56	3.24	*3.26 (2.22, 4.80)*
2005	0.55	0.12	*4.80 (0.44, 52.99)*	12.04	0.55	*22.00 (2.84, 170.41)*	17.99	11.08	*1.62 (0.76, 3.46)*	57.48	14.29	*4.02 (1.96, 8.25)*	49.24	7.23	*6.81 (2.22, 20.88)*	42.14	24.59	*1.71 (0.54, 5.40)*	10.78	3.13	*3.44 (2.31, 5.12)*
2006	0.54	0.23	*2.40 (0.34, 17.05)*	11.83	6.99	*1.69 (0.76, 3.78)*	55.72	23.31	*2.39 (1.49, 3.83)*	90.83	26.79	*3.39 (1.98, 5.79)*	48.37	19.54	*2.48 (1.11, 5.53)*	41.39	13.80	*3.00 (0.81, 11.17)*	17.07	6.59	*2.59 (1.94, 3.46)*
Overall IRR† and *p* for trend†																			***0.96***	***0.99***	***2.33 (1.68, 3.23)***

Table 4.4 (continued)

ER-/PR-	Urban-Rural Incidence and Incidence Ratios																				
	Age-Groups (Years)																		Overall		
	0-29			30-39			40-49			50-59			60-69			70+					
Years	Urban	Rural	*Urb-Rur IR (95% CI)*	Urban	Rural	*Urb-Rur IR (95% CI)*	Urban	Rural	*Urb-Rur IR (95% CI)*	Urban	Rural	*Urb-Rur IR (95% CI)*	Urban	Rural	*Urb-Rur IR (95% CI)*	Urban	Rural	*Urb-Rur IR (95% CI)*	Urban	Rural	*Urb-Rur IR (95% CI)*
2001	0.59	0.12	*4.80 (0.44, 52.99)*	4.68	3.51	*1.33 (0.38, 4.73)*	14.79	15.23	*0.97 (0.45, 2.10)*	16.03	8.33	*1.92 (0.62, 5.97)*	12.15	1.93	*6.28 (0.65, 60.42)*	0.00	3.76	*0.00*	4.07	2.78	*1.47 (0.87, 2.46)*
2002	0.29	0.36	*0.80 (0.08, 7.70)*	10.38	6.34	*1.64 (0.68, 3.95)*	14.57	5.00	*2.91 (1.06, 8.02)*	28.95	13.68	*2.12 (0.90, 4.98)*	7.98	11.42	*0.70 (0.14, 3.46)*	26.62	0.00	-	5.76	2.96	*1.95 (1.23, 3.09)*
2003	0.57	0.12	*4.80 (0.44, 52.99)*	6.80	6.80	*1.00 (0.38, 2.66)*	14.31	13.10	*1.09 (0.50, 2.41)*	28.44	17.47	*1.63 (0.73, 3.63)*	15.68	11.22	*1.40 (0.39, 4.95)*	8.72	0.00	-	5.20	3.89	*1.34 (0.86, 2.08)*
2004	0.28	0.35	*0.80 (0.08, 7.70)*	6.67	3.89	*1.71 (0.58, 5.10)*	19.67	13.66	*1.44 (0.71, 2.92)*	20.31	2.64	*7.70 (1.63, 36.25)*	23.08	9.18	*2.51 (0.77, 8.24)*	0.00	7.14	*0.00*	5.43	2.84	*1.91 (1.20, 3.03)*
2005	0.28	0.12	*2.40 (0.15, 38.41)*	4.38	2.74	*1.60 (0.43, 5.96)*	9.69	7.91	*1.22 (0.49, 3.22)*	17.49	14.29	*1.22 (0.47, 3.16)*	26.51	9.04	*2.93 (0.93, 9.24)*	33.72	10.54	*3.20 (0.72, 14.29)*	4.83	2.89	*1.67 (1.03, 2.72)*
2006	0.00	0.00	-	3.23	2.15	*1.50 (0.34, 6.70)*	17.67	10.10	*1.75 (0.81, 3.77)*	29.46	7.65	*3.85 (1.44, 10.25)*	37.21	5.33	*6.98 (1.92, 25.37)*	8.28	0.00	-	6.11	2.11	*2.89 (1.76, 4.75)*
Overall IRR† and *p* for trend†																			***0.81***	***0.76***	***1.72 (1.28, 2.32)***

*All incidences are per 100,000 women.

†Adjusted for age, stage and year of diagnosis.

ER+/PR- and ER-/PR+ categories had too few cases and were excluded from this analysis.

Figure 4.1. Age-specific urban-rural incidence of breast cancer by hormone receptor status in Gharbiah, 2001-2006. All incidences are per 100,000 women. There are 6 age-groups: 0-29, 30-39, 40-49, 50-59, 60-69 and 70 or more, each represented by a point on the graph for each year in that sequence.

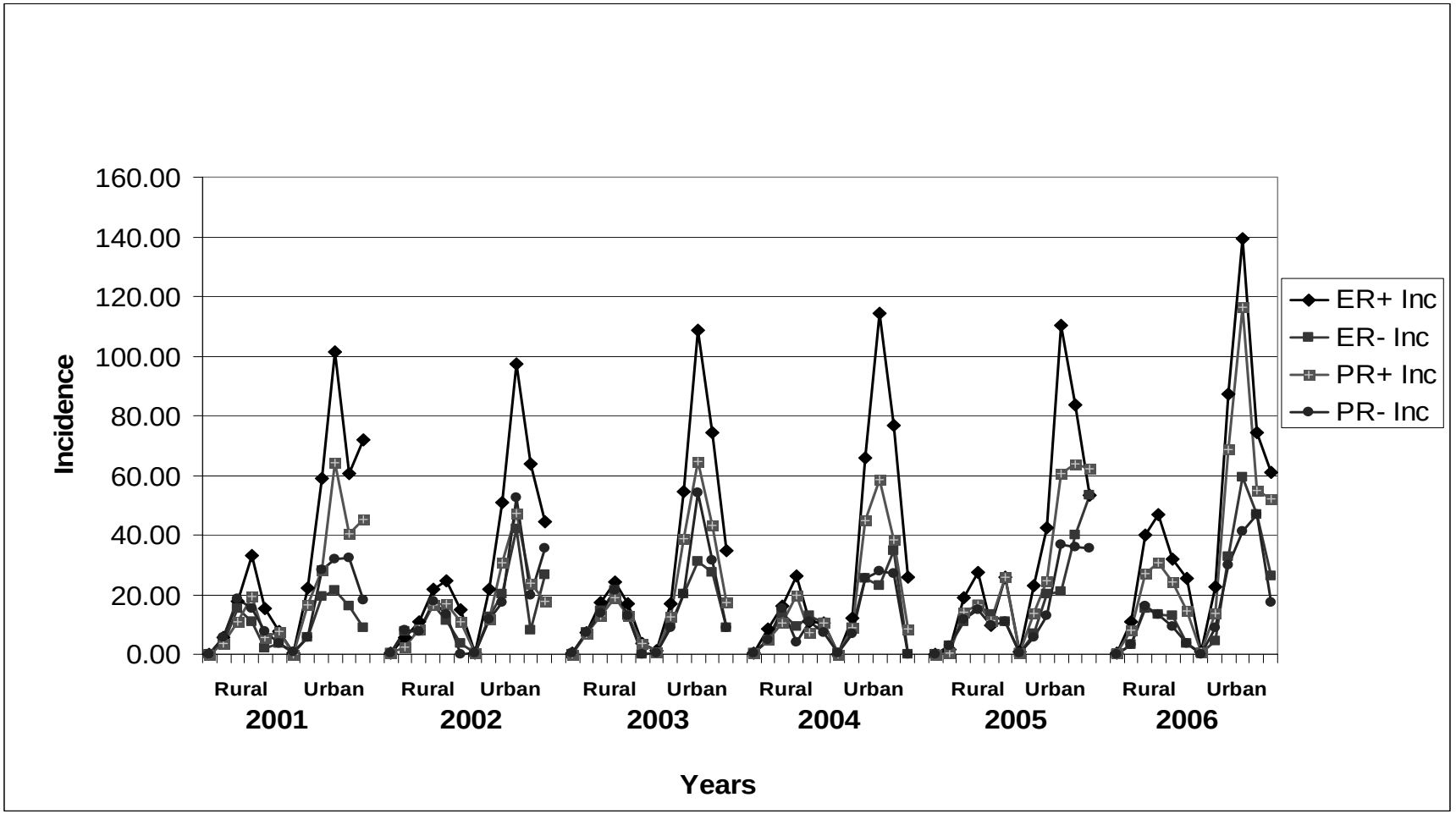
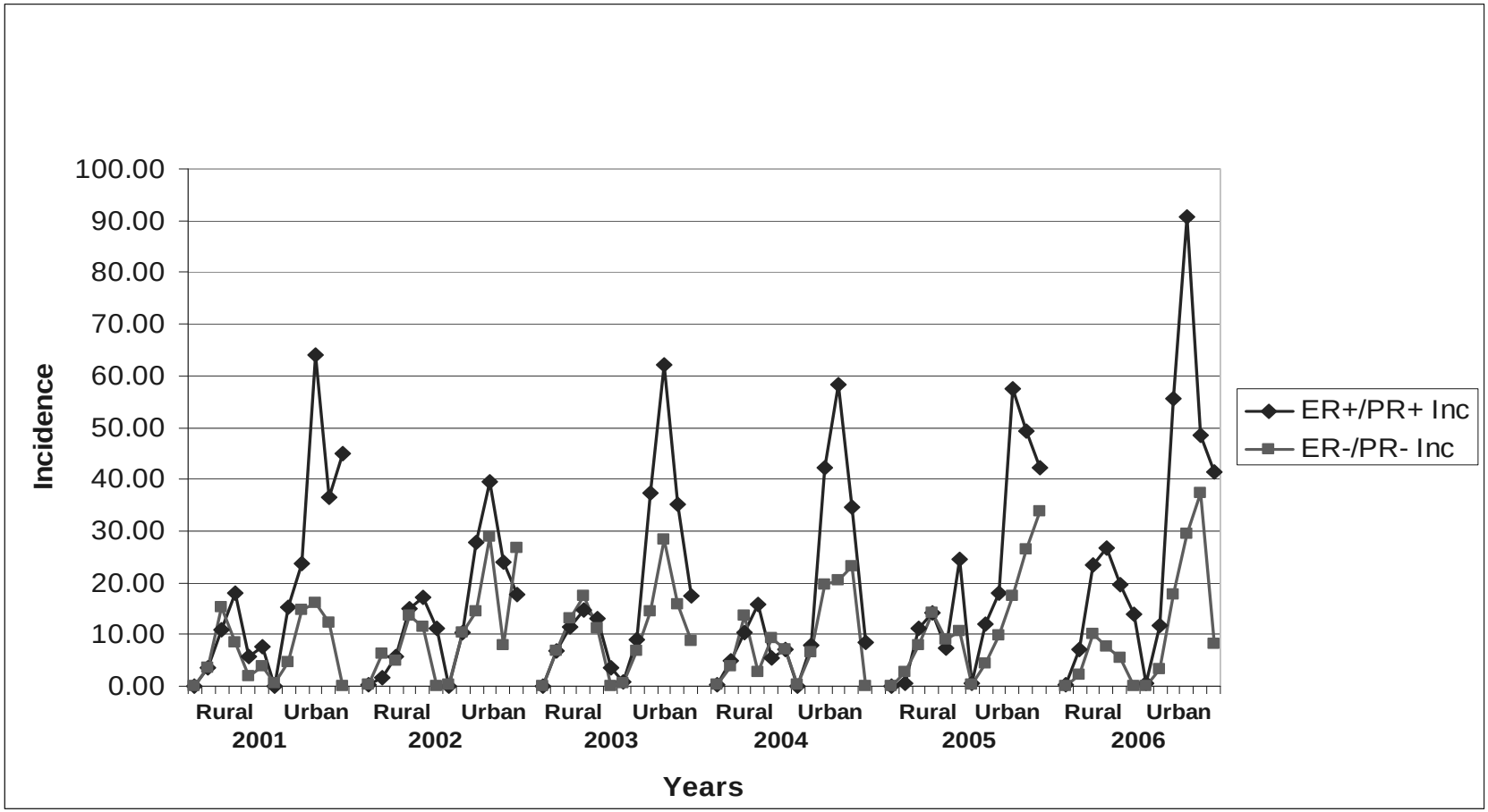


Figure 4.2. Age-specific urban-rural incidence of breast cancer by joint hormone receptor status in Gharbiah, 2001-2006. All incidences are per 100,000 women. There are 6 age-groups: 0-29, 30-39, 40-49, 50-59, 60-69 and 70 or more, each represented by a point on the graph for each year in that sequence. ER+/PR- and ER-/PR+ categories had very few cases and were excluded from this graph.



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CHAPTER V

URBAN-RURAL DIFFERENCES IN FEMALE MALIGNANCIES IN EGYPT (1999-2002)

INTRODUCTION

Malignancies specific to female organs such as those of breast, uterus, and ovary tend to have a hormone-related etiology [1-3]. Reproductive risk factors that increase the exposure of women to higher levels of endogenous estrogens seemingly lead to an increased risk of such cancers [1-3]. The malignancies of these three organs also have higher incidence rates in more affluent or developed countries compared to the developing world [4]. However, among these three organ sites, breast cancer is the most common cancer with the highest incidence in most populations across the world [4,5]. This difference in incidence between various organ sites may be due to differences in tissue structure of the organs and their anatomical site and/or physiological function which translates into differences in exposure.

Cervical cancer is also a malignancy that is specific to women but has a risk profile and epidemiology quite unlike that of breast, ovary or uterus. Cervical cancer has mainly an infectious etiology and the human papillomavirus (HPV) is found implicated in most

cases of cervical cancer [6]. As is true for most infectious diseases, cervical cancer has higher incidence in developing and more tropical countries.

Within developing countries, urban areas tend to be more affluent and developed compared to the rural areas. This difference in development and industrialization translates into differences in exposure to certain man-made chemicals called xenoestrogens that have been shown to act like natural hormones within the body and have been implicated in numerous *in vitro*, animal studies and human studies to increase the risk of breast cancer [7]. Numerous studies across the world have shown that xenoestrogen presence and exposure is higher in urban areas of the world [8-14]. Over the past several years, we explored the differences between developed and developing populations with a special focus on Egypt where distinct differences between urban and rural areas exist [15,16] and may provide a unique setting for investigating the association between development and urbanization and differences in cancer incidence and distribution.

In our previous studies in Gharbiah, Egypt, we found 3-4 times higher incidence of breast cancer and estrogen receptor positive breast tumors in urban areas than in rural areas [In press]. However, because of the hormonal etiology of breast cancer and the likelihood that populations in urban areas might be exposed to xenoestogenic compounds, like in other urban areas in other countries [8-14], we hypothesized that the incidence of other gynecological malignancies such as uterus and ovary must be higher in urban populations. At the same time higher xenoestrogen exposure must not have any effect on

creating urban-rural differences for cervical cancer which does not have a hormonal etiology. Thus, we examined the hypothesis that the incidence of uterine and ovarian cancer is higher in urban areas as compared to rural areas while the incidence of cervical cancer is not significantly different between urban and rural areas in Gharbiah, Egypt. For these purposes we analyzed the data from the population-based Gharbiah Cancer Registry for the four year-period of 1999-2002 to assess differences in urban-rural incidence of uterine, ovarian, and cervical cancers.

METHODS

The methods of this study are similar to the methods published before [17]. Here we have provided the methods specific to this study in brief.

Study Population

The study population consisted of all women diagnosed with uterine, ovarian, and cervical cancer from 1999 to 2002, a total period of four years, in the Gharbiah population based cancer registry, Tanta, Egypt. Cases' registry number, age at diagnosis, address, address code, smoking status, occupation, basis of diagnosis, tumor grade, stage, morphology, medical record number and place of reference were abstracted from routinely collected registry data. Use of human subject data was approved by the University of Michigan Institutional Review Board and the Gharbiah Cancer Center Ethics Committee and the data was stripped of all personal identifiers as instructed.

Gharbiah Population-Based Cancer Registry

The Gharbiah population-based cancer registry was founded in 1998 as a part of the Middle East Cancer Consortium (MECC) and is located in Tanta, the capital of Gharbiah Province [18]. This is an active registry and it collects cases from a number of sources in the governorate to determine cancer incidence. Most of the breast cancer cases came from Tanta Cancer Center (40-50%) and Gharbiah Cancer Society (20-25%). The remaining cases came from pathology laboratories (10%), Mansoura Radiotherapy Hospital (3-4%), Insurance hospitals (4-5%), NCI, Cairo (2-3%) and mortality records (4-5%). Most of the cases are diagnosed by pathological confirmation [19]. The World Health Organization (WHO) ICD-9 coding is used to determine the types of cancer. Cases were registered with SEER staging information from 1999-2002 although all available records for patients from 1999-2002 was retrieved and previous SEER staging was replaced by AJCC staging.

Gharbiah Province

Gharbiah Province is an administrative region located 90 kilometers north of Cairo in the Nile Delta Region. It has eight districts with Tanta being the capital of Tanta district as well as of the entire governorate. Gharbiah has a population of more than 4 million people and 49% of them are women. Approximately, 30% of the population resides in urban areas and almost 47% of the female population is below the age of 20 according to the 2006 Central Agency for Public Mobilization and Statistics (CAPMAS) census. Most of the residents residing in rural areas are part of an agricultural economy but most people living in cities participate in industrial occupations with most of the industries located in the two of the largest districts of Tanta and El Mehalla.

Census Data

Census data for female population in Gharbiah was obtained from the 1996 and 2006 CAPMAS census [20] and constant growth of the population was assumed to project populations for the years in between using a linear regression model. The linear growth rates of eight districts were applied to the urban and rural populations within that district to determine urban and rural populations from 1999 through 2002. The census data consisted of 16 age categories at 5 year intervals. 6 age categories were created from these by collapsing the age categories below 29 years and by collapsing age categories in 10 years interval after that. These population figures formed the denominators to calculate the overall, age-specific, district specific and urban-rural incidence rates for ovarian, uterine and cervical cancer in women.

Urban Rural Classification

The urban rural classification followed the CAPMAS coding (need to get CAPMAS definition of urban and rural) of urban and rural areas. Urban areas consisted of all the capital cities of the eight districts of the governorate while the remaining areas in the governorate were considered rural. Each case in the registry is assigned a residence code based on their residential address that follows the CAPMAS coding. This code was used to classify patients as urban or rural.

Statistical Analyses

Descriptive statistics and rate analyses were completed using SAS (Ver. 9; SAS Institute, Cary, NC). Univariate analyses were used to develop a descriptive profile using demographic and geographical indicators. Yearly raw and age adjusted incidence rates for breast cancer were calculated for Gharbiah governorate, each of the eight districts and urban and rural areas for the governorate and each district. Age-specific rates - overall and urban-rural, were calculated for each of 6 age categories. Raw incidence rates were calculated by taking the number of cases per year (1999 through 2002) divided by the person-year estimates for 1999 to 2002. Direct age-adjusted incidence rates were calculated by direct age-standardization for each district and their urban and rural areas using world population as the standard [4]. We also compared world age-standardized overall and urban-rural incidence rates to US SEER incidence rates. Incidence Rate Ratios (IRRs) and p-values for trend were calculated using negative binomial regression by the GENMOD procedure in SAS. Age, histology and stage at diagnosis could have been potential confounders. However, histology was uniform in distribution across urban-rural strata and stage at diagnosis did not affect IRs by more than 10%. Therefore we have reported age-standardized IRs and 95% confidence intervals.

As additional analyses following our initial results, we also compared urban-rural incidence of female leukemia (a cancer with mostly genetic and some environmental etiology and thus will most likely have least differences between urban and rural populations), all female cancers except breast and uterine cancer (two cancers with maximal links to hormonal risk factors in addition to other factors), all female cancers (including breast and uterine cancer) and hormonal cancers (breast and uterus).

RESULTS

Number of cases was highest for ovarian cancer followed by uterine and cervical cancer respectively (Table 5.1). More cases for all three cancer sites came from urban areas than from rural areas for all the three cancer sites (73.19% for uterus, 53.62% for ovary, and 58.25% for cervix). The number of cases of ovarian and uterine cancers were fairly constant across the years from 1999-2002. There was some variation seen in the number of cervical cancer cases with only 13 cases seen in 2001 while 38 cases were registered in 2000. For most organ sites and for most ages, the number of urban cases was higher except for ovarian cancer (1999 and 0-29 age category). Among districts, most cases for all cancer sites came from Tanta, the largest district. Most of the cases were diagnosed microscopically.

Crude incidence per 100,000 women for all three cancers was low with cervical cancer having the lowest incidence (Uterus – 1.91, Ovary – 3.83, Cervix – 1.43) (Table 5.2). However, urban incidence of all three cancers was higher than rural incidence – the highest difference being seen for uterine cancer (IRR = 6.07, 95% CI = 4.17, 8.85). Age-standardized rates for all cancer sites were much lower than US SEER (white) rates for all three cancer sites.

Overall age-specific incidence of the three cancers shows that these are diseases of old age and the incidence increases with increasing age (Table 5.3). A peak in incidence was seen for the age group of 50-59 years for ovarian and 60-69 years for uterine and cervical cancers. A comparison of age-specific urban and rural incidence shows some interesting features for all three cancers. For all cancer sites, urban

incidence is higher than rural incidence from an early age and urban incidence keeps increasing with age with highest incidence seen at 70+ age category for urban areas except for cervical cancer. Rural incidence, however, peaks at 60-69 years and then declines. The differences in incidence between urban and rural areas are much wider for uterine cancer in most age-groups compared to other two cancer sites with the highest difference seen at the 70+ age-group (IRR = 14.39, 95% CI = 4.24, 48.87) (Table 5.3).

Among the districts, Tanta has the highest incidence for all three cancer sites (Table 5.4). For ovary the incidence in Tanta is slightly higher compared to Basyoon (IRR = 1.56, 95% CI = 0.45, 5.49) while incidence is almost similar in the other districts. For uterine and cervical cancers the incidence in Tanta is much higher compared to Basyoon (Uterus – IRR = 4.14, 95% CI = 0.41, 42.04; Cervix – IRR = 11.31, 95% CI = 0.15, 867.4). However, due to very low number of cases these estimates have large standard errors.

We also looked at female leukemia and other female cancers in groups to observe any gradients in terms of urban-rural differences in incidence in Gharbiah (Table 5.5). We found that leukemia had the lowest urban-rural differences (overall IRR = 2.24) followed by all female cancers except those with most pronounced hormonal etiology (breast and uterus) (overall IRR = 2.81). Finally, when we included the cancer sites with hormonal etiology, the urban-rural incidence difference increased further (overall IRR = 3.50). Looking at only hormonal cancer sites (breast and uterus) the urban-rural incidence was much higher than any other cancer groups (overall IRR = 4.96) (Table 5.5).

DISCUSSION

This study showed a higher incidence of uterine, ovarian, and cervical cancers in urban than in rural areas in the Gharbiah Province of Egypt. Furthermore, the most striking finding was the almost 6- times higher incidence of uterine cancer in urban areas than in rural areas of Gharbiah. We also found a gradient of increasing urban-rural difference for all female cancers. Cancers such as leukemia with mainly genetic and some environmental risks (which will likely lead to minimal differences between urban and rural populations) had the lowest IRR followed by urban-rural IRR seen for female cancers except cancers with hormonal malignancies. On including cancers with hormonal malignancies in the group analyses the IRR increased by almost 70%. This urban-rural difference increased further by 146% when we looked at only hormonal cancers. In our previous studies we have found 3-4 times higher incidence of breast cancer and estrogen receptor positive (ER+) breast cancer in urban areas of Gharbiah Province [17]. These urban-rural differences seen for breast cancer in addition to a 6 times higher incidence of uterine cancer in urban areas clearly show that women in urban areas experience a much higher exposure to hormonal risk factors of cancers.

In preparation for fertilization, the uterus undergoes cyclical changes every month mainly under the influence of estrogen. Thus, uterine muscle which is rich in estrogen receptors shows highest proliferation rate during the first 18 days of menstrual cycle [21]. The "unopposed estrogens" hypothesis (long-term exposure to estrogens, not counterbalanced by the presence of progesterone) is the most widely accepted hypothesis on the etiology of endometrial cancer [21]. Given the fact that urban and rural women in Egypt are

genetically similar and most of the risk factors of uterine cancer being environmental, it can be inferred that urban women in Egypt have a higher exposure to environmental estrogens compared to rural women. It is clear from large surveys in Egypt that differences in reproductive factors are not substantial between urban and rural women [22]. Also, given the fact that oral contraceptive use (which is protective for uterine cancer and is most likely to be used by urban women) is quite low among Egyptian women [22], there are probably other environmental estrogenic factors that are leading to higher urban incidence of uterine cancer.

Xenoestrogens are such factors whose presence and exposure is much higher in urban areas than in rural areas, a fact that has been seen in many populations across the world [8-14]. Given the high rate of development of urban centers of Egypt, the exposure of women in urban Egypt might be high to xenoestrogens. There have been very few studies looking at the effect of xenoestrogens on uterine cancer in humans. However, animal studies show clearly that xenoestrogens are quite capable of causing uncontrolled uterine proliferation usually through the same pathways via which endogenous estrogens act [23,24]. There are more studies related to breast cancer and xenoestrogens and we have already hypothesized that higher urban incidence of breast cancer is possibly due to higher exposure to xenoestrogens [25].

Obesity is the other leading risk factor of uterine cancer worldwide and has been known to explain 40% of endometrial cancer incidence [26]. However, the differences between urban and rural women in terms of obesity are minimal [22] especially in Lower Egypt

and this cannot possibly explain the large urban-rural differences in uterine cancer incidence. Also, it is quite likely that uterine bleeding, the only way in which uterine cancer is detected is much easily detectable in urban areas. However, primary healthcare coverage in rural Egypt is 100% [27] and the remotest rural area in Gharbiah is not more than 50 kilometers away from the capital city of the province. Thus, access to healthcare in Gharbiah is not an issue and will not affect detection of rural cases. Also, the coverage of the Gharbiah registry is quite high and given the multiple quality checks in the registry it is unlikely that rural cases of uterine cancer are being missed.

Nevertheless, we saw around two times higher incidence of ovarian cancer and leukemia in urban areas and almost three times higher incidence of cervical cancer in urban areas in Gharbiah. Apart from differences in urban-rural distribution of risk factors, there might still be slight differences in healthcare access and behavior between urban and rural areas responsible for higher urban incidence of female cancers. In terms of etiology, ovary is really not a hormonally related cancer since it is not under direct stimulatory effects of estrogen. Ovarian cancer development is more related to risk factors that lead to chronic inflammation related to 'incessant ovulation' [28,29]. Thus the observation of lack of any large urban-rural differences with regards to ovarian cancer is explainable. Cervical cancer on the other hand is a cancer much closely related to sexual behavior than other cancers [6]. Cervical cancer detection is also related to access of women to gynecological clinics and pap smears and as such a higher urban incidence is possible. However, cervical cancer has a very low incidence in Egypt and given the

low number of cases it is much more difficult to draw clear inferences regarding this site in the context of our study.

Overall, we found approximately 6-times higher incidence of uterine cancer in urban areas in this study and in addition to the evidence from our recent studies which showed an almost 4 times higher urban incidence of breast cancer and ER+ breast cancer [17]. Thus, it is likely that women in urban areas have higher exposure to environmental hormonal risk factors, possibly xenoestrogens. This is especially so in the light of any substantial differences between urban and rural women with regards to known risk factors of uterine and breast cancer. Xenoestrogens are a preventable cause of cancer and more research at the individual level is required to clearly enumerate a possible association between xenoestrogens with uterine and breast cancers.

Table 5.1. Characteristics of cases with uterine, ovarian and cervical cancer by urban-rural status in Gharbiah, Egypt from 1999-2002.

Variable	Descriptive Category	Uterus		Ovary		Cervix	
		Urban No. (%)	Rural No. (%)	Urban No. (%)	Rural No. (%)	Urban No. (%)	Rural No. (%)
Total Cases		101 (73.19)	37 (26.81)	148 (53.62)	128 (46.38)	60 (58.25)	43 (41.75)
Year of Diagnosis	1999	17 (62.96)	10 (37.04)	29 (46.03)	34 (53.97)	15 (53.57)	13 (46.43)
	2000	27 (72.97)	10 (27.03)	34 (55.74)	27 (44.26)	25 (65.79)	13 (34.21)
	2001	24 (68.57)	9 (25.71)	38 (50.67)	37 (49.33)	9 (56.25)	7 (43.75)
	2002	33 (80.49)	8 (19.51)	47 (61.04)	30 (38.96)	11 (52.38)	10 (47.62)
Age	0-29	1 (100)	0	16 (45.71)	19 (54.29)	1 (100)	0
	30-39	4 (100)	0	26 (63.41)	15 (48.39)	5 (62.50)	3 (37.50)
	40-49	16 (66.67)	8 (33.33)	33 (55.00)	27 (45.00)	11 (50.00)	11 (50.00)
	50-59	30 (68.18)	14 (31.82)	34 (47.22)	38 (52.78)	18 (60.00)	12 (40.00)
	60-69	32 (72.73)	12 (27.27)	26 (55.32)	21 (44.68)	20 (64.52)	11 (35.48)
	70+	18 (85.71)	3 (14.29)	13 (61.91)	8 (38.10)	5 (45.46)	6 (54.54)
District	Tanta	51 (80.95)	12 (19.05)	67 (62.62)	40 (37.38)	30 (69.77)	13 (30.23)
	El-Mehalla	25 (71.43)	10 (28.57)	39 (59.09)	27 (40.91)	19 (73.08)	7 (26.92)
	Kafr El-Zayat	6 (66.67)	3 (33.33)	10 (52.63)	9 (47.37)	3 (50)	3 (50)
	Zefta	8 (66.67)	4 (33.33)	10 (58.82)	7 (41.18)	1 (25)	3 (75)
	Samanoud	2 (100)	0	5 (41.67)	7 (58.33)	2 (28.57)	5 (71.43)
	El Santa	3 (60)	2 (40)	7 (25.93)	20 (74.07)	2 (28.57)	5 (71.43)
	Kotoor	3 (37.5)	5 (62.5)	3 (30)	7 (70)	2 (25)	6 (75)
	Basyoon	3 (75)	1 (25)	7 (38.89)	11 (61.11)	1 (100)	0
Basis of Diagnosis	Microscopic	90 (72)	35 (28)	132 (54.1)	112 (45.9)	59 (58.42)	42 (41.58)
	Non-microscopic	1 (100)	0	7 (30.44)	16 (69.56)	1 (100)	0
	Death-Certificate	10 (83.33)	2 (16.67)	9 (100)	0	0	1 (100)

Table 5.2. Overall, urban and rural incidence, crude and age-standardized to the world population, and urban-rural incidence rate ratios for uterus, ovary and cervix in Gharbiah, Egypt.

Organs	Incidence (per 100,000 women) and Incidence Rate Ratios (IRRs)								
	Crude*	**Crude Urban**	**Crude Rural**	**IRR (95% CI)**	**ASW†**	**ASW Urban**	**ASW Rural**	**ASW IRR**	**ASW US SEER (White)**
Uterus	1.91	4.52	0.74	6.07 (4.17, 8.85)	2.94	6.63	1.17	5.68	18.4
Ovary	3.83	6.62	2.57	2.57 (2.03, 3.26)	5.02	8.15	3.50	2.33	13.2
Cervix	1.43	2.68	0.86	3.11 (2.10, 4.59)	2.09	3.68	1.31	2.80	6.8

*Crude = Crude incidence rate; †ASW = Age-standardized to the world population

Table 5.3. Overall and urban-rural age-specific incidence rates* of uterine, ovarian and cervical cancers in Gharbiah, Egypt.

Age-Groups	Uterus				Ovary				Cervix			
	Overall	**Urban**	**Rural**	**IRR (95% CI)**	**Overall**	**Urban**	**Rural**	**IRR (95% CI)**	**Overall**	**Urban**	**Rural**	**IRR (95% CI)**
0-29	0.02	0.07	0.00	-	0.77	1.19	0.59	2.02 (1.04, 3.93)	0.02	0.07	0.00	-
30-39	0.39	1.18	0.00	-	4.03	7.66	2.21	3.47 (1.84, 6.55)	0.79	1.47	0.44	3.33 (0.80, 13.95)
40-49	3.25	5.95	1.70	3.50 (1.50, 8.17)	8.12	12.28	5.75	2.14 (1.29, 3.55)	2.98	4.09	2.34	1.75 (0.76, 4.03)
50-59	10.12	20.17	4.89	4.12 (2.19, 7.78)	16.55	22.86	13.28	1.72 (1.08, 2.73)	6.90	12.10	4.19	2.89 (1.39, 5.99)
60-69	14.53	32.60	5.84	5.59 (2.88, 10.85)	15.52	26.49	10.21	2.59 (1.46, 4.61)	10.24	20.38	5.35	3.81 (1.83, 7.95)
70+	14.01	40.81	2.84	14.39 (4.24, 48.87)	14.01	29.47	7.56	3.90 (1.62, 9.41)	7.34	11.34	5.67	2.00 (0.61, 6.55)

*All incidence rates are per 100,000 women

Table 5.4. Incidence rates* and incidence rate ratios (IRRs) of uterine, ovarian and cervical cancer by districts of Gharbiah, Egypt.

Districts	Uterus		Ovary		Cervix	
	Incidence	IRR (95% CI)	Incidence	IRR (95% CI)	Incidence	IRR (95% CI)
Tanta	3.68	4.14 (0.41, 42.04)	6.25	1.56 (0.45, 5.49)	2.51	11.31 (0.15, 867.37)
El-Mehalla	1.89	2.12 (0.17, 26.46)	3.56	0.89 (0.21, 3.71)	1.40	6.31 (0.07, 555.26)
Kafr El-Zayat	1.30	1.47 (0.10, 21.78)	2.75	0.69 (0.15, 3.20)	0.87	3.92 (0.04, 414.13)
Zefta	1.55	1.75 (0.13, 23.73)	2.20	0.55 (0.11, 2.85)	0.52	2.33 (0.02, 336.7)
Samanoud	0.37	0.42 (0.01, 19.27)	2.23	0.56 (0.11, 2.87)	1.30	5.85 (0.06, 527.23)
El Santa	0.76	0.85 (0.04, 18.29)	4.09	1.02 (0.26, 4.06)	1.06	4.77 (0.05, 462.93)
Kotoor	1.51	1.70 (0.12, 23.36)	1.88	0.47 (0.08, 2.67)	1.51	6.79 (0.08, 584.62)
Basyoon	0.89	1.00	4.00	1.00	0.22	1.00

*All incidence rates are per 100,000 women

Table 5.5. Incidence rates* and incidence rate ratios (IRRs) of female cancers by groups in Gharbiah, Egypt.

Sites	1999			2000			2001			2002			Overall		
	Urban	Rural	IRR	Urban	Rural	IRR	Urban	Rural	IRR	Urban	Rural	IRR	Urban	Rural	IRR
Female Leukemia	6.94	3.12	2.22	5.04	2.67	1.89	6.77	2.55	2.65	6.30	2.83	2.23	6.26	2.79	**2.24**
All female cancers except breast and uterus	96.98	34.44	2.82	96.67	33.53	2.88	96.91	36.25	2.67	103.71	35.91	2.89	98.61	35.05	**2.81**
All female cancer sites	175.70	48.83	3.60	170.48	47.54	3.59	171.03	53.25	3.21	185.91	51.24	3.63	175.84	50.24	**3.50**
Breast and uterus	75.25	14.39	5.23	72.73	14.09	5.16	71.62	16.61	4.31	81.15	15.56	5.22	75.22	15.17	**4.96**

*All incidence rates are per 100,000 women

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CHAPTER VI

CONCLUSIONS

In spite of years of progress in detection and treatment of breast cancer it still remains a disease on the rise, especially in the developing world. In addition to all the efforts that have been directed towards early detection and better treatment of breast cancer, it is also imperative that we understand the risk factors and the disease process of breast cancer development. This understanding will enable us to effectively institute ways of primary prevention of this most common cancer of women. This dissertation was directed towards understanding the differences in breast cancer incidence among urban and rural populations in Egypt which might be differentially exposed to one of the less well-studied risk factors of breast cancer such as xenoestrogens. These urban-rural differences may be analogous to international differences we see between developed and developing countries. Understanding the reasons for these differences was the main objective of this dissertation. In the process we also managed to discover similar urban-rural differences for other malignancies e.g. uterine cancer.

Chapter II brought together the diverse parts of the background research – differences in breast cancer incidence and hormone receptor specific breast cancer incidence in space and time, inability of known risk factors of breast cancer in explaining those differences, possibility of xenoestrogens in being the risk factors that could explain the incidence differences and how all of these are linked to stem cells and possible

critical periods of exposure to various risk factors. This led to our hypotheses about breast cancer incidence and hormone receptor positive breast cancer incidence being higher in urban areas of developing countries since urban women might be more exposed to xenoestrogens. These hypotheses in this form have never been put forth before according to our knowledge.

This part of the dissertation and what we investigated following it also effectively addressed one of the limitations that might be hindering the study of the association of xenoestrogens and breast cancer. Most of the studies in the past looking at the link of xenoestrogens with breast cancer were done in developed countries where the exposure to xenoestrogens is widespread. Thus, at the population level there has been a lack of unexposed populations. However, through our hypotheses and subsequent study results we have shown clearly that the links between xenoestrogens and breast cancer might be studied more effectively if such studies are conducted in developing countries which have urban and rural populations.

Chapter III consisted of the study we did following the hypotheses that had been enumerated in Chapter II. We looked at urban and rural differences in breast cancer incidence across eight years from 1999 to 2006. For all those years we found consistent results in terms of urban incidence of breast cancer being three to four times higher than rural incidence. These differences were quite consistent across age-groups and even at the district level, more developed districts showed higher incidence of breast cancer. These differences could not be explained through known differences in risk factors of breast cancer at the population level nor could these differences be accounted for by differences in health access and behavior. One of the possible differences between urban

and rural women which led to high incidence of breast cancer in urban areas were differences in possible exposure to environmental estrogenic risk factors such as xenoestrogens.

Although we had a lack of information on individual level of exposure of women with regards to xenoestrogens, existing knowledge on xenoestrogens from previous studies in other countries clearly shows their presence and exposure to be higher in urban areas and developed countries, as illustrated in Chapter III. Thus, in this study we observed the effects of a probable higher xenoestrogen presence in urban areas as we had hypothesized earlier.

As a part of Chapter IV we further explored our hypotheses focused on hormone receptor specific breast cancer incidence for six years: from 2001 to 2006 in the same population in which we had discovered a 3-4 times higher incidence of breast cancer in urban areas. Our hypothesis was that we will observe a higher hormone receptor positive breast cancer incidence in urban areas. Our results clearly supported our hypothesis and we saw a 2-4 times higher incidence of ER+ and ER+/PR+ in urban areas. We found these results to be consistent across the six years of our study and for all age-groups. This further strengthened the premise that urban women were having a higher exposure to estrogenic risk factors. Since we had shown in the previous Chapter that known risk factors of breast cancer (which represent endogenous sources of estrogen) could not explain higher breast cancer incidence in urban areas, probably it was exogenous sources of estrogen like xenoestrogens whose exposure was higher in urban areas.

We also discovered a higher incidence of ER- breast cancer in urban areas although ER- breast cancer incidence was less than ER+ breast cancer incidence within

urban areas. This observation could be explained very clearly in terms of stem cell theory of cancer and critical periods of exposure. Overall, we were able to enumerate clearly the hypotheses we had laid out in Chapter II.

As a part of Chapter V, we explored the urban-rural differences in female malignancies further since if urban women had higher exposure to estrogenic risk factors, possibly xenoestrogens, this would have translated into higher urban incidence of malignancies in other sites as well which were end organs for estrogenic influences. And quite in line with our hypotheses we found five to six times higher incidence of uterine cancer in urban areas compared to rural areas. In terms of being an end organ for estrogen effects uterus is much more sensitive than breast. Although we don't have studies in humans linking xenoestrogen exposure with uterine cancer, in animals studies xenoestrogens have been shown to cause high proliferation of the endometrium. Taking our analyses further, we also found a gradient of urban-rural differences for female malignancies with breast and uterus showing the highest urban incidence as compared to other cancers. This may suggest that urban women had higher exposure to hormonal or estrogenic risk factors, probably xenoestrogens.

Thus as part of this humble effort in further exploring a less well-studied risk factor of breast cancer, namely xenoestrogens, we were able to highlight possible effects of a higher presence of environmental exposures such as xenoestrogens in urban women in Egypt. Since xenoestrogens are linked with development and multiple studies have shown their presence and exposure to be higher in urban areas and developed countries, our findings are a small but significant step in the direction of further enumerating the possible associations between environmental exposures such as xenoestrogens and breast

cancer as well as uterine cancer. Some of the strengths of this dissertation emanate from our ability to show results that were in agreement with our hypotheses. Moreover these results were consistent across the years we investigated. The data we used was from a population-based registry, the GPCR, which is quite a credible registry. Being part of regular quality control checks from IARC, NCI and Emory University, the data we used for our analyses was of very high quality having very few errors. We also looked at a large sample size especially for the study in Chapter III which involved almost 5000 breast cancer cases. Also, Gharbiah province has a limited number of pathological laboratories which provided added credibility to our results in the study in Chapter IV.

However, this dissertation has limitations as well, the biggest being the lack of information at the individual level regarding known risk factors of breast cancer as well as xenoestrogens. We were also limited in our analyses by the absence of some cases in the registry for the years 2003 – 2006 and by the absence of ER and PR information of the cases since these were not routinely collected as a part of the registry. However, we did made all possible attempts to retrieve all available records and to obtain all possible information on these missing cases. Also, additional analyses showed that in spite of these missing cases our analyses and conclusions were still valid. Another possible limitation arises from the reduced ability to replicate this study in other developing countries and cancer registries due to probable lack of urban-rural classification as was seen in GPCR.

As a part of future directions for research following the findings in this dissertation we definitely need to address the most important limitation mentioned above. A well designed study looking at urban-rural differences in known risk factors of breast

and uterine cancer and xenoestrogen levels will provide the much needed confirmation of our results which open pathways for many such studies in this population in Egypt. We also need studies in this population to assess urban-rural differences in health access and behaviors. Also, studies to investigate probable time periods of xenoestrogen exposure in female infants and adolescents will pave way for in-depth research into molecular methods of assessing breast cancer risk. We also need to conduct further similar urban-rural comparisons in other developing countries to confirm our findings. This could involve conducting similar research with population-based registries in other developing countries. Using health indicators of the countries we can decide if the registries may have valid data and furthermore data from valid registries could be used to make urban-rural and inter-registry comparisons regarding cancer rates for various sites.

In conclusion, xenoestrogens are man-made chemicals and as such their presence and use can be regulated. In addition to further research in the appropriate populations using better methods we also need better control and regulation of xenoestrogens which will be quite in conformity with the "precautionary principle". This will help us better understand the etiology of breast cancer. Furthermore, it will also translate into primary prevention of breast as well as uterine cancer, with the impact being greater for breast cancer. I look forward to continuing the work I started at my doctoral level so that I can pursue further the goals of better understanding mechanisms of cancer causation while at the same time advocating ways of cancer prevention and control.

APPENDIX

RISK FACTORS ACCORDING TO ESTROGEN RECEPTOR STATUS OF BREAST CANCER PATIENTS IN TRIVANDRUM, SOUTH INDIA

INTRODUCTION

Estrogen receptor (ER) status of breast tumors has been instrumental in defining an important subtype of breast cancer with differences observed in risk factors, treatment and prognosis [1-7]. Numerous studies in the past have looked at differences in etiology and risk factors pertaining to presence or absence of ER-alpha. Most of these studies were conducted in Western populations as early as 1980s [1-5]. Around the same time it was also discovered that ER+ tumors that lacked progesterone receptor (PR) expression were less responsive to endocrine therapy compared to tumors that expressed PR [8]. This led to studies in the past decade that looked at the link of various risk factors of breast cancer and combined ER/PR information to better explain the underlying differences between the various subtypes of breast cancer [9-13]. Chen *et al* [14] have emphasized the importance of taking into account the ER/PR status information of breast tumors both for effective treatment as well as risk prediction for instituting prophylactic measures. Although there might be numerous ways to subtype breast cancer, the classification into ER+ and ER- cancer remains a key divider [14]. However, information related to ER status is lacking for populations in developing countries. In

fact, in most developing countries determination of hormone receptor status is not a part of standard protocol for treatment of breast cancer despite the fact that the Breast health global initiative classified hormone receptor status determination as a basic level therapy in the treatment of breast cancer [15,16].

India is one such developing country where breast cancer is the most common cancer among women in most parts, mainly in the urban areas [17]. Despite this there have been very few studies on breast cancer in India. Most studies that have looked at hormone receptor status in the recent past utilized secondary data and explored associations with limited number of clinical variables [18,19]. This has prevented effective extrapolation of those results at the population level. Indeed, there have been hardly any studies in India that have looked at the association of hormone receptor status of breast cancer and the underlying risk factors. In this paper we present the results from a case-control study that was conducted in Trivandrum, Kerala. This study was done as a part of a multi-center breast cancer study in collaboration with International Agency for Research on Cancer (IARC) in South Asia. The main objective of the study was to look at the urban-rural differences between determinants of breast cancer to gain a broad understanding of breast cancer risk factors in India. We hypothesized that the known relationships of risk factors with ER status must hold true in this region of the world as well since evidence indicates that factors that increase exposure to estrogens increase the propensity of ER+ breast cancer occurrence [12]. We were also in a unique position to explore a few additional risk factors due to the unique composition of the population in this study with presence of various religions and mostly rural subjects.

METHODS

Study setting, subject recruitment and confounding variables

Between 2002 and 2005, the study was conducted at the Regional Cancer Center (RCC), Trivandrum in the state of Kerala. The cases (n=1208) were women with histologically confirmed incident primary breast cancer who attended the above hospital. All cases with past history of any cancer except non-melanoma skin cancer were excluded from the study. 20 cases had incomplete data and were excluded from analyses. In addition 288 cases did not have ER data and were also excluded for the purposes of this study providing a total of 900 cases. The controls (n= 1208) were subjects who did not have cancer and accompanied cancer patients other than those with breast cancer attending the same hospital during the same time period, and matched to cases by age ($\pm$5 years), and residence status (urban/rural). The RCC institutional review board approved the study. Written informed consent was obtained from all participants. The participation rates were more than 90% for both cases and controls.

In-person interview of each case and control was conducted at the above hospital using a pre-tested structured questionnaire at the time of admission to the study. Information on demographic and socio-economic variables, reproductive history, time spent in household activities on a normal day, residential history, occupational history, personal, and family medical history, tobacco and alcohol habits, and diet history were collected by trained interviewers. Anthropometric measurements were taken at the end of interview. Hormone receptor status was obtained from the medical records. All subjects were asked to list all places of residence where they had lived for at least one year, starting with the place of birth. Urban/rural residence status was collected

according to the definition of national census. If the subject lived in a 'Panchayat', residence status is defined as 'rural' and all other areas such as 'municipality' and 'corporation' are defined as 'urban'.

Socio-economic status (SES) was assessed by using independent scores given to yes and no questions related to home ownership, availability of toilet and running water as well as possession of comfort/luxury items such as electrical/ gas stove, refrigerator, TV, air conditioner, car, motorcycle/scooter, bicycle, and computer owned by the subjects. These scores were summed up to create a SES score which was proxy for the income level of the women.

Anthropometric measurements

The height (in cm) and weight (in kg) of each case and control were measured using standard equipments. All subjects were asked to remove their shoes before measurements were taken. In addition to this, weight was measured with light clothing. All measurements were done twice in succession and averaged for a final value. Body mass index (BMI: kg/m^2) was computed as weight in kilogram divided by height in meters squared. Three mutually exclusive BMI groups were created based on the tertile distribution of BMI of all subjects since there were very few obese subjects in this study ($BMI \geq 30$).

ER status determination process

Representative section of formalin fixed paraffin embedded tumor tissue is stained immunohistochemically using estrogen receptor (Clone 1D5-Dakocytomation). Both the

intensity and extent of staining (as denoted by brown staining of nuclei) is determined and scored 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong) positivity.

Data Analyses

All statistical analyses were performed using SAS 9.1.3 (SAS Institute Inc., Cary, NC). To estimate the association of various risk factors and the ER status of breast cancer we used unconditional logistic regression. Three way analyses were conducted: Case-case analysis comparing ER+ and ER- cases, ER+ cases and controls and ER- cases and controls. The case-case analysis points towards presence of heterogeneity between the two case subgroups whereas the comparison between each case subgroup and controls allows for deriving risk estimates for determinants of breast cancer [12]. We also further extended our analyses by stratifying it based on menopausal status of subjects since most of the previous studies have suggested that risk profiles for breast cancer differ between pre- and post menopausal women. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived from the fitted models to reflect risk factor-ER status associations.

The following reproductive, demographic and lifestyle factors were used for analysis: urban or rural status - self-reported, age (in years) divided into three categories (<35, 35-50, >50), religion (Hindu, Christian or Muslim), marital status (married versus unmarried, divorced or separated), education (college or higher education versus less than college education), SES Score (low or high), BMI (1^{st} tertile ≤21.4, 2^{nd} tertile >21.4 to ≤25.1 and 3^{rd} tertile >25.1), age at menarche (<13, 13-16, >16), parity (nulliparous, 1-4 children, 5 or more children), age of marriage (<18, 18-21, 21-24 and >24), total duration of breast feeding (<36 months, 36-54 months, 54-78 months, >78 months), and total

amount of physical activity per day (<3 hours, 3-4 hours, 5-6 hours and >6 hours). SES Score was dichotomized using the median value and the categories for the last three variables were determined by dividing their distributions into quartiles followed by comparison of the higher three categories with the lowest quartile. We also looked at a number of other variables in our models during the preliminary analysis which included marital status, use of oral contraceptive pills or hormone replacement therapy, family history of breast cancer, smoking and alcohol intake. However, these factors did not affect the associations in the underlying model and the results that have been presented here are for variables defined by using the fewest categories having relevant associations.

RESULTS

Characteristics of the study population

Among the cases (n=1188), ER status information was not available for 288 cases and these were excluded from analysis. Among the 900 cases that were included in our analysis 323 cases were ER+ and 577 cases were ER-. We compared the baseline factors for excluded and included cases and found the distribution of these factors to be similar in the two groups. Overall, it can be inferred from the distribution among the controls that the study population was predominantly rural (80.1%), Hindu (65.9%), with less than college education (87.5%), low SES score (72.1%) and was married (86.6%) with one to four children (84.7%) (Table I). Most of the population was premenopausal (67.1%) and had got married between 18-24 years (61.7%) having breastfed for a total of 78 months or less (73.4%). Most women were also quite active throughout the day for 5 hours or more (72.8%) and had low prevalence of using oral contraceptive pills (3.4%) (Table I).

Risk factor association outcomes based on ER status

Case-case analysis showed appreciable results for two variables (Table II). ER+ status of breast cancer was negatively associated with Muslim religion when compared to Hindus (OR = 0.59, 95% CI = 0.37, 0.93) and breastfeeding for 54-78 months was negatively associated with ER+ breast cancer (OR = 0.61, 95% CI = 0.39, 0.94). On comparing with controls these associations became more prominent with Muslim women having positive association with ER- breast cancer (OR = 1.49, 95% CI = 1.09, 2.03) and increased duration of breastfeeding being protective for ER+ and ER- breast cancer with a stronger protective effect seen for ER+ breast cancer (p for trend = 0.004). Women with higher SES score had a positive association with both ER+ and ER- breast cancer when compared to controls but positive associations with ER+ breast cancer were stronger (OR = 1.43, 95% CI = 1.07, 1.92). Case-case analysis also showed an inverse association of ER+ breast cancer with increasing BMI, an effect which can be seen prominently in the positive association seen in comparison of ER- cases with controls (p for trend <0.001) (Table II). Increasing age of marriage increased the probability of having both ER+ and ER- breast cancer with significant p-values of trend for both. However, the positive association of age of marriage more than 24 years was higher for ER- breast cancer (OR = 2.01, 95% CI = 1.42, 2.87). Increased duration of physical activity was protective for both ER+ and ER- breast cancer (p for trend for both < 0.0001) (Table II).

On stratifying by menopausal status the negative association of Muslim religion with ER+ breast cancer was prominently limited to premenopause as was seen from the effects

in case/case analysis (OR = 0.45, 95% CI = 0.23, 0.89) and ER-/control analysis (OR = 1.87, 95% CI = 1.27, 2.79) (Table III). Women with higher SES score had a positive association with having ER+ breast cancer mainly in postmenopausal women (OR = 1.60, 95% CI = 1.03, 2.47). The positive relationship of ER- breast cancer with increasing BMI was seen clearly only in premenopausal women (p for trend <0.001). Increasing age of marriage increased the odds of having both ER+ and ER- breast cancer in both premenopausal women and postmenopausal women although in both groups this association was stronger for ER- breast cancer with strongest association seen with ER- breast cancer in premenopausal women married above 24 years of age (OR = 2.50, 95% CI = 1.49, 4.10) (Table III). Breastfeeding appeared protective for ER+ breast cancer in both premenopausal and postmenopausal women and ER- breast cancer only among premenopausal women. Increasing physical activity was protective for both ER+ and ER- breast cancer among both premenopausal and postmenopausal women. This protection was seen maximally for postmenopausal women who were active for more than 4 hours, mainly for ER+ breast cancer with appreciable results seen for physical activity of 5-6 hours (OR = 0.60, 95% CI = 0.36, 0.98) (Table III).

DISCUSSION

In this study on breast cancer conducted in South India among mostly rural women we found a high proportion of ER- cases. Muslim women had a higher likelihood of developing ER- breast cancer, an effect most clearly observed in premenopausal period. Women with higher SES had more likelihood of developing ER+ breast cancer. Increasing BMI increased the likelihood of ER- breast cancer mainly in premenopausal

women. Age of marriage was positively associated with both ER+ and ER- breast cancer, although the effects were stronger for ER- breast cancer among premenopausal women. Increased breastfeeding and physical activity were in general protective for both ER+ and ER- breast cancer. However, protective effects for breastfeeding were stronger for ER+ breast cancer premenopausally while protective effects of physical activity were stronger for ER+ breast cancer postmenopausally.

The results of this study differ from those of other similar studies from European and North American populations since the majority of cases in this study were ER- as compared to breast cancer cases reported in western parts of the world where the majority of cases are ER+. This is consistent with the findings from previous studies done in India which also found a very high proportion of ER- cases [19]. Similar results have also been seen from other countries in Asia such as Pakistan [20], China [21], and Japan [22]. One of the reasons put forth earlier for this observation has been younger age at presentation among Indian women [19], although this may not be the only factor responsible. Another factor that might be affecting this shift in proportion of cases is perhaps a reduced exposure to exogenous estrogens such as hormone replacement therapy (HRT) and oral contraceptive pills (OCPs), which leads to a higher occurrence of ER- tumors as compared to ER+ tumors [23]. It has been seen that Indian women prefer long term methods of contraception such as tubal ligation rather than oral contraception [24]. According to the Indian National Family Health Survey (NFHS), the oral contraceptive pill usage among rural women in Kerala is only 0.6% [25]. The NFHS also shows that reproductive factors in rural Indian women still favor a reduced exposure to endogenous estrogens which will further keep the proportion of ER+ tumors low [25].

India is secular country and different religions have varying lifestyles, customs and traditions. One of the most interesting findings of this study is that ER- status of breast cancer was associated with being a Muslim compared to a Hindu. Redkar *et al* [18] looked at religious differences in ER status of breast cancer in the past and reported that Muslims had the lowest proportion of ER+ breast cancer when compared to other religions. However in that study they had not controlled for any of the confounders and could not lucidly explain their finding. It is known from previous studies that breast cancer due to causes that act through mechanisms that are independent of hormonal exposures tends to be ER- [12]. Among genetic risk factors BRCA1 tumors tend to be ER- than ER+ [26]. Muslims all over the world including India are known to favor consanguineous marriages. Among Indian Muslims hailing from Kerala the prevalence of consanguinity is quite low (9.4%) when compared to other parts of India but this is still higher than other communities. For Indian Muslims overall, the prevalence of consanguinity is as high as 22% [27]. Studies done in other parts of the Indian subcontinent, mainly Pakistan, have shown that consanguinity is a risk factor for breast cancer due to the inheritance of breast cancer susceptibility genes [28]. Liede *et al* [29] found significant associations of consanguinity with early onset breast cancer in the Pakistani population and have proposed that recessive genes might play a role in the etiology of breast cancer. The association of genetic risk factors of breast cancer with ER- tumors might explain the high proportion of ER- tumors among Muslim women in India as well. However, it is imperative that this finding be explored further in populations from other parts of India. ER- tumors are more aggressive, non-responsive

to endocrine therapy and have a higher tendency to relapse early and Muslim women in India might bear a disproportionately high burden of disease due to this.

This study found an association of ER+ status of breast cancer higher SES score mainly in postmenopausal women. These findings are consistent with findings in numerous previous studies that have looked at SES as a risk factor and have found higher risk of breast cancer with higher SES [30-34]. It has been speculated that higher SES is related to and may be a proxy for other factors related to nutrition and physical activity [32] which change the internal hormonal milieu and increase a woman's lifetime exposure to estrogen which translates into increased occurrence of ER+ breast cancer mainly in the postmenopausal period.

The positive association of increasing BMI with ER- breast cancer, mainly observed among premenopausal women was peculiar and interesting. In the past, most studies in Asian as well as Western populations have found positive associations of BMI with risk of ER+ status of breast cancer among postmenopausal women [9,35]. Among premenopausal women, positive association [30], no association [36-39] or inverse associations [40-42] have been seen for breast cancer risk and increasing BMI. However the most recent WCRF report [43] suggests that body fatness is protective for breast cancer in premenopausal women. It is quite possible that the factors which lead to an inverse risk of breast cancer in premenopausal women might be related to the positive association between ER- breast cancer and increasing BMI among premenopausal women who develop breast cancer. Obesity can result in decreased circulatory estrogen levels causing anovulatory cycles [44,45]. In addition, obesity also leads to a state of relative insulin resistance, chronic hyperinsulinemia, and an increase in IGF-1 bioactivity

because of insulin-mediated decreases in IGF-binding protein 1 (IGFBP-1) and IGFBP-2. Insulin has been shown to be a growth factor for breast cancer cells, and level of C-peptide, a marker of hyperinsulinemia and insulin resistance predicted breast cancer risk [46]. Meta-analysis of prospective studies for IGF-1 found a positive association with risk for premenopausal, but not postmenopausal, breast cancer [47]. Thus, breast cancer in premenopausal women is most likely caused by non-estrogenic influences which results in ER- breast cancer.

Marriage in this population of predominantly rural women was closely associated to having children and breastfeeding which get postponed due to a later age of marriage. This was clear from the correlation observed between age of first childbirth and age of marriage. Both pregnancy and breastfeeding have long-term protective effects against breast cancer because of the increased differentiation of breast tissue under the effect of female hormones – mainly progesterone [48-51]. Increased age of marriage must lead to a lack of differentiation in the breast tissue making it more susceptible to harmful effects of non-estrogenic mutagens as well as genotoxic effects of estrogen which has been known to cause ER- breast cancers as well [52,53]. Moreover, being married and having children might also reduce the level of circulating hormones or increase the levels of sex hormone binding globulin [54-56]. This result is consistent with the results of Lord *et al* who also found an increased risk of ERPR- breast cancer with late age at first birth [57].

In this population where the frequency of breastfeeding was high, cumulative breastfeeding was seen to provide protection for ER+ breast cancer for all women and ER- breast cancer only in premenopausal women. Evidence of protective effects of breastfeeding is inconsistent in studies in western populations probably due to low

prevalence of breastfeeding [58]. Increased breastfeeding has been speculated to protect the breast against cancer through a number of mechanisms which more prominently include excretion of carcinogens in breast milk and increased differentiation of breast tissue [58]. Breast milk has been known to carry a number of lipid soluble chemicals that can act as mammary carcinogens [59-61]. Also, increased breastfeeding leads to increased differentiation of breast tissue [62] and both these mechanisms might be playing a big role in protecting the breast from both ER+ and ER- breast cancers.

More than 80 studies looking at the association of physical activity and breast cancer have found physical acitivity to have a protective effect [43,63]. This protective effect is due to a multitude of factors including reduction in circulating levels of and cumulative exposure to sex steroid hormones, changes to insulin-related factors and adipocytokines, modulation of inflammation and immune system and hormonal and cellular metabolism pathways [63]. In this study, this protection was seen most prominently if a woman is active for 5 hours or more per day and was almost similar for both ER+ and ER- breast cancer. In comparison of premenopausal and postmenopausal women stronger dose-response was seen in postmenopausal women for both ER+ and ER- breast cancer, a finding consistent with the WCRF report [43]. Also, Enger *et al* found similar results showing decreased breast cancer risk with increased physical activity across all ER/PR categories for both premenopausal and postmenopausal women [64].

Although the above study had a large number of participants which made the estimates in this study quite powerful there might have been a few sources of bias. One of them could have arisen from the differences between participants and non-participants in the study. However, response rates were high (90%) and it is unlikely that participants

and non-participants would have differed significantly. Among the participants, only 900 cases were chosen for analysis since ER status information was lacking for rest of the 288 cases. Both, the included and excluded cases had similar distributions of baseline factors and thus it is unlikely it would have biased our results. Also, this study was a hospital based case-control study which might generate different forms of bias arising from controls being similar to cases, and recall bias. However, we ensured that none of the controls were relatives of breast cancer patients. In addition, most of the items in the questionnaire included questions on lifestyle and reproductive factors which were not difficult to recall. Thus, any bias arising due to a hospital-based design seems to be minimal.

One of the main strengths of this study was the ability to disentangle the effect of ER status from that of menopause on breast cancer. The results might suggest independent effects of ER status and menopausal status on the association between breast cancer and the various risk factors (BMI, parity etc.). However, one of the most important things that was lacking in this study was the lack of information on PR status of breast tumors. Given the increasing emphasis on better classification of breast tumors on joint hormone receptor status, the presence of this information might have made the findings of this study more meaningful. Nevertheless, PR expression depends on ER expression and as such ER status of tumors is good predictor of PR status. Overall, the findings of this study are quite significant in better understanding of breast cancer in the context of India and other developing countries. It is also essential that hormone receptor status determination be made a routine part of the breast cancer treatment in developing countries since it would optimize the use of endocrine therapy and chemoprevention

agents by improving their cost: benefit ratio. This would reduce the economic burden of breast cancer in developing countries quite effectively.

Table I – Distribution of characteristics for women in the case-control study, Kerala, India.

	Cases (n = 900)								
	ER+ (n = 323)		ER- (n = 577)		ER+case/ER-case	Controls (n = 1208)		ER+ case/controls	ER-case/controls
	No.	%	No.	%	OR (95% CI)*	No.	%	OR (95% CI)†	OR (95% CI)*
Urban-rural status									
Rural	243	75.23	447	77.47	1.00	968	80.13	1.00	1.00
Urban	80	24.77	130	22.53	1.13 (0.82, 1.56)	240	19.87	1.33 (0.99, 1.77)	1.17 (0.92, 1.49)
Age (years)									
<35	36	11.15	69	11.96	1.00	265	21.94	1.00	1.00
35-50	159	49.23	314	54.42	0.97 (0.62, 1.52)	705	58.36	1.66 (1.13, 2.45)	1.71 (1.27, 2.30)
>50	128	39.63	194	33.62	1.27 (0.80, 2.01)	238	19.70	3.96 (2.63, 5.96)	3.13 (2.26, 4.34)
Religion									
Hindu	225	69.66	360	62.39	1.00	797	65.98	1.00	1.00
Muslim	32	9.91	92	15.94	0.56 (0.36, 0.86)	161	13.33	0.93 (0.69, 1.27)	1.27 (0.95, 1.68)
Christian	66	20.43	125	21.66	0.85 (0.60, 1.19)	250	20.70	0.70 (0.47, 1.06)	1.11 (0.86, 1.42)
Education									
Less than college	259	80.19	490	84.92	1.00	1058	87.58	1.00	1.00
College or higher	64	19.81	87	15.08	1.39 (0.98, 1.99)	150	12.42	1.74 (1.26, 2.41)	1.25 (0.94, 1.67)

Table I (continued)

	Cases (n = 900)								
	ER+ (n = 323)		ER- (n = 577)		ER+case/ER-case	Controls (n = 1208)		ER+ case/controls	ER-case/controls
	No.	%	No.	%	OR (95% CI)*	No.	%	OR (95% CI)†	OR (95% CI)*
SES Score									
Low	185	57.28	366	63.43	1.00	872	72.19	1.00	1.00
High	138	42.72	211	36.57	1.29 (0.98, 1.71)	336	27.81	1.94 (1.50, 2.50)	1.50 (1.21, 1.85)
Marital status									
Unmarried	12	3.72	11	1.91	1.00	17	1.41	1.00	1.00
Married	249	77.09	458	79.38	0.50 (0.22, 1.15)	1047	86.67	0.34 (0.16, 0.72)	0.68 (0.31, 1.46)
Others (Divorced, separated)	62	19.20	108	18.72	0.53 (0.22, 1.26)	144	11.92	0.61 (0.28, 1.35)	1.16 (0.52, 2.58)
BMI									
1st Tertile (≤21.4)	91	28.17	144	24.96	1.00	466	38.58	1.00	1.00
2nd Tertile (>21.4 to ≤25.1)	122	37.77	200	34.66	0.97 (0.68, 1.36)	395	32.70	1.58 (1.17, 2.14)	1.64 (1.27, 2.11)
3rd Tertile (>25.1)	110	34.06	233	40.38	0.75 (0.53, 1.06)	347	28.73	1.62 (1.19, 2.21)	2.17 (1.69, 2.79)
Age of menarche									
≤13	93	28.79	184	31.89	1.00	365	30.22	1.00	1.00
>13	230	71.21	393	28.79	0.86 (0.64, 1.16)	843	69.78	0.93 (0.71, 1.22)	1.08 (0.87, 1.34)
Menstrual status									
Premenopausal	153	47.37	316	54.77	1.00	811	67.14	1.00	1.00
Postmenopausal	170	52.63	261	45.23	1.35 (1.02, 1.77)	397	32.86	2.27 (1.77, 2.91)	1.69 (1.38, 2.07)

Table I (continued)

	Cases (n = 900)					Controls (n = 1208)			
	ER+ (n = 323)		ER- (n = 577)		ER+case/ER-case			ER+ case/controls	ER-case/controls
	No.	%	No.	%	OR (95% CI)*	No.	%	OR (95% CI)†	OR (95% CI)*
Parity									
>4 children	39	12.07	70	12.15	1.00	135	11.18	1.00	1.00
1-4 children	258	79.88	474	82.29	0.99 (0.65, 1.51)	1024	84.77	0.87 (0.60, 1.28)	0.88 (0.65, 1.20)
Nulliparous	26	8.05	32	5.56	1.48 (0.77, 2.83)	49	4.06	1.84 (1.01, 3.33)	1.24 (0.73, 2.11)
Age of Marriage									
<18 years	39	12.54	71	12.54	1.00	200	16.79	1.00	1.00
18-21 years	84	27.01	171	30.21	0.79 (0.51, 1.22)	455	38.20	0.79 (0.54, 1.15)	0.99 (0.73, 1.36)
21-24 years	84	27.01	143	25.27	0.94 (0.61, 1.47)	280	23.51	1.28 (0.86, 1.89)	1.35 (0.98, 1.87)
>24 years	104	33.44	181	31.98	0.92 (0.60, 1.41)	256	21.49	1.73 (1.81, 2.53)	1.87 (1.36, 2.57)
Total Duration of Breastfeeding									
<36 months	105	32.51	145	25.13	1.00	239	19.78	1.00	1.00
36-54 months	71	21.98	139	24.09	0.71 (0.48, 1.03)	313	25.91	0.52 (0.37, 0.73)	0.73 (0.55, 0.98)
54-78 months	64	19.81	156	27.04	0.57 (0.39, 0.83)	335	27.73	0.44 (0.31, 0.62)	0.77 (0.58, 1.02)
>78 months	83	25.70	137	23.74	0.84 (0.58, 1.21)	321	26.57	0.59 (0.42, 0.82)	0.70 (0.53, 0.94)

Table I (continued)

	Cases (n = 900)					Controls (n = 1208)			
	ER+ (n = 323)		ER- (n = 577)		ER+case/ER-case			ER+ case/controls	ER-case/controls
	No.	%	No.	%	OR (95% CI)*	No.	%	OR (95% CI)†	OR (95% CI)*
Physical Activity per Day									
< 3 hours	81	26.64	116	22.31	1.00	128	11.91	1.00	1.00
3-4 hours	69	22.70	97	18.65	1.23 (0.83, 1.83)	164	15.26	1.10 (0.76, 1.58)	0.89 (0.65, 1.22)
5-6 hours	96	31.58	206	39.62	0.81 (0.57, 1.14)	507	47.16	0.49 (0.36, 0.68)	0.61 (0.48, 0.79)
>6 hours	58	19.08	101	19.42	0.99 (0.66, 1.49)	276	25.67	0.55 (0.38, 0.79)	0.55 (0.41, 0.74)
Oral contraceptive pill usage									
No	317	98.14	558	97.04	1.00	1166	96.52	1.00	1.00
Yes	6	1.86	17	2.96	0.62 (0.24, 1.59)	42	3.48	0.53 (0.22, 1.25)	0.85 (0.48, 1.50)

*Unadjusted odds ratios and 95% confidence intervals.

Table II – Adjusted* odds ratios and 95% confidence intervals (CI) for the association between potential risk factors and breast cancer characterized by estrogen receptor (ER) status for women in case-control study, Kerala, India.

	ER+ case/ER- case (n = 323/577)		ER+ case/controls (n = 323/1208)		ER- case/controls (n = 577/1208)	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Age						
<35	1.00		1.00		1.00	
35-50	1.11	(0.69, 1.83)	1.66	(1.08, 2.56)	1.62	(1.17, 2.25)
>50	1.16	(0.62, 2.18)	3.07	(1.73, 5.45)	3.07	(1.95, 4.83)
Religion						
Hindu	1.00		1.00		1.00	
Muslim	0.59	(0.37, 0.93)	0.92	(0.66, 1.28)	1.49	(1.09, 2.03)
Christian	0.80	(0.56, 1.14)	0.83	(0.53, 1.28)	1.08	(0.82, 1.40)
Education						
Less than college	1.00		1.00		1.00	
College or higher	1.36	(0.90, 2.07)	1.41	(0.96, 2.08)	1.13	(0.81, 1.57)
SES Score						
Low	1.00		1.00		1.00	
High	1.24	(0.90, 1.70)	1.43	(1.07, 1.92)	1.11	(0.88, 1.41)

Table II (continued)

	ER+ case/ER- case (n = 323/577)		ER+ case/controls (n = 323/1208)		ER- case/controls (n = 577/1208)	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
BMI						
1st Tertile (≤21.4)	1.00		1.00		1.00	
2nd Tertile (>21.4 to ≤25.1)	0.89	(0.62, 1.28)	1.29	(0.94, 1.79)	1.46	(1.12, 1.90)
3rd Tertile (>25.1)	0.72	(0.50, 1.04)	1.27	(0.91, 1.79)	1.87	(1.43, 2.44)
	p for trend = 0.087		*p* for trend = 0.17		*p* for trend < 0.0001	
Age of menarche						
≤13	1.00		1.00		1.00	
>13	0.86	(0.63, 1.17)	1.07	(0.80, 1.43)	1.17	(0.93, 1.47)
Menstrual status						
Premenopausal	1.00		1.00		1.00	
Postmenopausal	1.27	(0.85, 1.88)	1.42	(0.98, 2.06)	1.06	(0.79, 1.42)
Parity						
Having children	1.00		1.00		1.00	
Nulliparous	1.09	(0.58, 2.06)	1.42	(0.78, 2.59)	1.21	(0.70, 2.08)

Table II (continued)

	ER+ case/ER- case (n = 323/577)		ER+ case/controls (n = 323/1208)		ER- case/controls (n = 577/1208)	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Age of Marriage						
<18 years	1.00		1.00		1.00	
18-21 years	0.89	(0.56, 1.42)	0.98	(0.65, 1.48)	1.12	(0.81, 1.56)
21-24 years	0.99	(0.61, 1.61)	1.55	(1.01, 2.39)	1.53	(1.08, 2.18)
>24 years	0.81	(0.50, 1.31)	1.60	(1.04, 2.48)	2.01	(1.42, 2.87)
	p for trend = 0.59		*p* for trend < 0.001		*p* for trend < 0.0001	
Total Duration of Breastfeeding						
<36 months	1.00		1.00		1.00	
36-54 months	0.75	(0.50, 1.15)	0.66	(0.44, 0.97)	0.83	(0.60, 1.15)
54-78 months	0.61	(0.39, 0.94)	0.52	(0.34, 0.77)	0.80	(0.58, 1.11)
>78 months	0.85	(0.54, 1.32)	0.58	(0.38, 0.87)	0.67	(0.47, 0.95)
	p for trend = 0.30		*p* for trend = 0.004		*p* for trend = 0.03	
Physical Activity per Day						
< 3 hours	1.00		1.00		1.00	
3-4 hours	1.23	(0.82, 1.85)	1.06	(0.72, 1.56)	0.91	(0.66, 1.28)
5-6 hours	0.86	(0.60, 1.23)	0.56	(0.40, 0.78)	0.68	(0.52, 0.88)
>6 hours	1.13	(0.73, 1.75)	0.72	(0.49, 1.07)	0.67	(0.49, 0.93)
	p for trend = 0.30		*p* for trend < 0.0001		*p* for trend < 0.0001	

* Adjusted for all the variables in this table

Table III – Adjusted* odds ratios and 95% confidence intervals (CI) for the association between potential risk factors and breast cancer characterized by estrogen receptor (ER) status among post and pre-/perimenopausal women in the case-control study, Kerala, India.

	OR (95% CI)					
	Postmenopausal			Premenopausal		
	ER+case/ER- case (n = 170/261)	ER+ case/controls (n = 170/397)	ER- case/controls (n = 261/397)	ER+case/ER- case (n = 153/316)	ER+ case/controls (n = 153/811)	ER- case/controls (n = 316/811)
Age (years)†	1.01 (0.98, 1.04)	1.06 (1.03, 1.09)	1.06 (1.03, 1.09)	1.02 (0.98, 1.06)	1.07 (1.04, 1.10)	1.05 (1.03, 1.08)
Religion						
Hindu	1.00	1.00	1.00	1.00	1.00	1.00
Muslim	0.78 (0.39, 1.51)	0.90 (0.47, 1.72)	1.20 (0.71, 2.02)	0.45 (0.23, 0.89)	0.81 (0.43, 1.51)	1.87 (1.27, 2.79)
Christian	0.75 (0.45, 1.24)	0.88 (0.53, 1.45)	1.28 (0.85, 1.92)	0.90 (0.54, 1.50)	0.93 (0.59, 1.46)	0.93 (0.65, 1.33)
Education						
Less than college	1.00	1.00	1.00	1.00	1.00	1.00
College or higher	1.35 (0.65, 2.81)	1.26 (0.58, 2.73)	1.27 (0.62, 2.60)	1.40 (0.83, 2.36)	1.57 (0.98, 2.51)	1.18 (0.80, 1.72)
SES Score						
Low	1.00	1.00	1.00	1.00	1.00	1.00
High	1.12 (0.72, 1.75)	1.60 (1.03, 2.47)	1.30 (0.90, 1.89)	1.40 (0.87, 2.24)	1.27 (0.83, 1.93)	0.93 (0.67, 1.28)

Table III (continued)

	OR (95% CI)					
	Postmenopausal			Premenopausal		
	ER+case/ER-case (n = 170/261)	ER+ case/controls (n = 170/397)	ER- case/controls (n = 261/397)	ER+case/ER-case (n = 153/316)	ER+ case/controls (n = 153/811)	ER- case/controls (n = 316/811)
BMI						
1st Tertile (≤21.4)	1.00	1.00	1.00	1.00	1.00	1.00
2nd Tertile (>21.4 to ≤25.1)	1.16 (0.69, 1.95)	1.72 (1.04, 2.84)	1.35 (0.88, 2.07)	0.72 (0.44, 1.20)	1.04 (0.66, 1.62)	1.53 (1.08, 2.16)
3rd Tertile (>25.1)	0.95 (0.56, 1.62) *p* for trend = 0.78	1.34 (0.81, 2.23) *p* for trend = 0.32	1.51 (0.98, 2.30) *p* for trend = 0.07	0.54 (0.32, 0.92) *p* for trend = 0.24	1.29 (0.80, 2.08) *p* for trend = 0.30	2.21 (1.54, 3.16) *p* for trend < 0.0001
Age of menarche						
≤13	1.00	1.00	1.00	1.00	1.00	1.00
>13	0.72 (0.45, 1.15)	0.93 (0.59, 1.48)	1.19 (0.81, 1.73)	1.02 (0.67, 1.56)	1.27 (0.86, 1.87)	1.21 (0.90, 1.63)
Parity						
Having children	1.00	1.00	1.00	1.00	1.00	1.00
Nulliparous	0.73 (0.29, 1.86)	1.12 (0.41, 3.07)	1.54 (0.62, 3.84)	1.56 (0.62, 3.88)	1.75 (0.81, 3.79)	1.07 (0.53, 2.18)

Table III (continued)

	OR (95% CI)					
	Postmenopausal			Premenopausal		
	ER+case/ER-case (n = 170/261)	ER+ case/controls (n = 170/397)	ER- case/controls (n = 261/397)	ER+case/ER-case (n = 153/316)	ER+ case/controls (n = 153/811)	ER- case/controls (n = 316/811)
Age of Marriage						
<18 years	1.00	1.00	1.00	1.00	1.00	1.00
18-21 years	1.03 (0.56, 1.88)	1.11 (0.64, 1.95)	1.15 (0.72, 1.84)	0.75 (0.35, 1.61)	0.89 (0.46, 1.70)	1.16 (0.72, 1.87)
21-24 years	0.93 (0.49, 1.77)	1.61 (0.87, 2.95)	1.54 (0.91, 2.61)	0.93 (0.43, 2.02)	1.58 (0.83, 3.00)	1.65 (1.00, 2.73)
>24 years	0.89 (0.47, 1.68)	1.34 (0.73, 2.49)	1.51 (0.89, 2.56)	0.65 (0.30, 1.42)	1.71 (0.89, 3.28)	2.50 (1.49, 4.10)
	p for trend = 0.88	*p* for trend = 0.08	*p* for trend = 0.05	*p* for trend = 0.35	*p* for trend = 0.005	*p* for trend < 0.0001
Total Duration of Breastfeeding						
<36 months	1.00	1.00	1.00	1.00	1.00	1.00
36-54 months	0.68 (0.35, 1.34)	0.52 (0.26, 1.02)	0.85 (0.46, 1.57)	0.84 (0.48, 1.46)	0.75 (0.46, 1.23)	0.81 (0.55, 1.20)
54-78 months	0.47 (0.24, 0.91)	0.44 (0.23, 0.85)	0.93 (0.52, 1.67)	0.75 (0.42, 1.35)	0.55 (0.32, 0.93)	0.70 (0.47, 1.04)
>78 months	0.72 (0.38, 1.33)	0.47 (0.25, 0.88)	0.61 (0.34, 1.10)	0.92 (0.44, 1.90)	0.50 (0.27, 0.95)	0.63 (0.40, 1.01)
	p for trend = 0.31	*p* for trend = 0.03	*p* for trend = 0.07	*p* for trend = 0.52	*p* for trend = 0.009	*p* for trend = 0.03

Table III (continued)

	OR (95% CI)					
	Postmenopausal			Premenopausal		
	ER+case/ER- case (n = 170/261)	ER+ case/controls (n = 170/397)	ER- case/controls (n = 261/397)	ER+case/ER- case (n = 153/316)	ER+ case/controls (n = 153/811)	ER- case/controls (n = 316/811)
Physical Activity per Day						
< 3 hours	1.00	1.00	1.00	1.00	1.00	1.00
3-4 hours	1.40 (0.82, 2.41)	1.37 (0.81, 2.33)	0.92 (0.57, 1.50)	1.05 (0.54, 2.03)	1.01 (0.55, 1.86)	1.05 (0.66, 1.67)
5-6 hours	0.83 (0.50, 1.38)	0.60 (0.36, 0.98)	0.70 (0.46, 1.05)	0.90 (0.53, 1.55)	0.62 (0.38, 1.01)	0.74 (0.51, 1.06)
>6 hours	0.93 (0.46, 1.86)	0.56 (0.29, 1.09)	0.60 (0.35, 1.03)	1.39 (0.75, 2.60)	1.00 (0.59, 1.71)	0.78 (0.52, 1.19)
	p for trend = 0.38	*p* for trend = 0.003	*p* for trend = 0.009	*p* for trend = 0.84	*p* for trend = 0.05	*p* for trend = 0.02

*Adjusted for all the variables in this table.
†Age has been used as a continuous variable.

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