AMERICAN UNIVERSITY OF BEIRUT

ASSOCIATION OF RELATIVE TELOMERE LENGTH IN PERIPHERAL BLOOD IN CONTROL SUBJECTS WITH THE CONCENTRATION OF BISPHENOL-A IN URINE AND IN PATIENTS WITH BREAST CANCER

by
FATIMA MOHAMAD SLEIMAN

A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science
to the Department of Pharmacology and Toxicology
of the Faculty of Medicine
at the American University of Beirut

Beirut, Lebanon
April 2016

AMERICAN UNIVERSITY OF BEIRUT

ASSOCIATION OF RELATIVE TELOMERE LENGTH IN PERIPHERAL BLOOD IN CONTROL SUBJECTS WITH THE CONCENTRATION OF BISPHENOL-A IN URINE AND IN PATIENTS WITH BREAST CANCER

by

FATIMA MOHAMAD SLEIMAN

Approved by:

Dr. Nathalie Zgheib, MD. Department of pharmacology and toxicology	Advisor
Dr. Ramzi Sabra, MD, MHPE. Department of pharmacology and toxicology	Member of Committee
Dr. Joseph Semaan, MD. Department of pharmacology and toxicology	Member of Committee
Dr. Rihab Nasr, PhD. Department of Anatomy, Cell Biology and Physiology	Member of Committee
Dr. Arafat Tfayli, MD. Department of Internal Medicine, Division of Hematology Oncology	Member of Committee

Date of thesis Defense: April 21, 2016

AMERICAN UNIVERSITY OF BEIRUT

THESIS, DISSERTATION, PROJECT RELEASE FORM

Student Name: _____
Last First Middle

◯ Master's Thesis ◯ Master's Project ◯ Doctoral Dissertation

☐ I authorize the American University of Beirut to: (a) reproduce hard or electronic copies of my thesis, dissertation, or project; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes.

☐ I authorize the American University of Beirut, **three years after the date of submitting my thesis, dissertation, or project,** to: (a) reproduce hard or electronic copies of it; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes.

_____

Signature Date

ACKNOWLEDGEMENTS

First of all, I want to thank Dr. Nathalie Zgheib, my amazing advisor, for her full support, advice and guidance that helped me in the completion of my thesis project. Thank you doctor for listening, understanding and helping me in every aspect throughout my project. You taught me how to be professional and appreciate the work I'm doing.

Second of all, I take this opportunity to thank Dr. Ramzi Sabra for the full support he provided me from the moment I started my graduate studies at AUB. You were always there to listen to our suggestions and complains and provide us with the best education and research environment that I'm so grateful for.

I am so thankful to all of my committee members for giving me the pleasure to review my thesis and providing me with their advice and guidance that I so appreciate.

I would like to show my biggest appreciation to my lovely department of pharmacology and toxicology. Chairperson, professors, doctors and staff members, I'll never forget you for the amazing support and the lovely environment you provided me with.

And to my second family at the lab T-352, Nahed Mogharbil, Reem Akika, Zainab Awada, Zainab Mhaidly and Sehrish Abbasi. I cherish our joyful moments we shared and I want to thank you for your endless help and support. We together make an amazing team.

Nahed, you were like a mom to me, you never failed to help me and solve my obstacles. Reem Akika you are amazing and thank you for your help and support. Zainab my lovely friend, I can't wait to attend your PhD defense, you are amazing and you supported me all the way. Zainab Mheidly, I'll never forget our memories that you managed to print and hang on the fridge. Sehrish although I didn't get to see you often, but I know that you are a wonderful person and I wish all the best for you.

I want to thank Dr. Hani Tamim as a PI in the BPA cohort and for helping us in the statistical analyses, and thanks to Dr. Mouneimne for the BPA measurement from urine samples.

I want to thank my family: Dad, Mom, my sister Sara and my brother Mahdi. You are the reason of my success and without your uttermost support and endless love I would never have been able to reach this far. I love you so much and I hope I made you proud.

Finally, to my other half, I want to thank you Jacob for your love and support that overwhelmed me throughout my thesis project. Thank you so much for always being there for me.

AN ABSTRACT OF THE THESIS OF

Fatima Mohamad Sleiman for Master of Science
Major: Pharmacology and Therapeutics

Title: Association Of Relative Telomere Length In Peripheral Blood In Control Subjects With The Concentration Of Bisphenol-A In Urine And In Patients With Breast Cancer

Background: BPA (Bisphenol-A) is an estrogen-like plastic monomer found ubiquitously in our environment. It can enter our body through different routes (oral, pulmonary and dermal) and from different sources. Very few studies were done in vitro to check the effect of BPA on telomere length, however no clinical studies were done to study the association between human exposure to Bisphenol-A and relative telomere length. Telomere length plays a critical role in maintaining genomic stability. Previous studies have shown an association between telomere length and different diseases and cancer types including breast cancer. However, different clinical studies on the association between telomere length and breast cancer risk have shown contradicting results.

Aims: We aimed to study the association between urinary BPA levels and relative telomere length (RTL) in peripheral blood samples of a large Lebanese cohort. In addition to that, we wanted to explore any difference in the mean relative telomere length in the peripheral blood of non-breast cancer and breast cancer females. Moreover, we wanted to investigate the association between other variables such as age and BMI with relative telomere length in peripheral blood.

Methods: After signing an informed consent, 501 Lebanese volunteers, of whom 319 were females, were recruited from Greater Beirut between February and June 2014. A fasting urine sample was collected in a glass container to avoid any exogenous BPA contamination. Peripheral whole blood was collected as well and stored along with the urine samples at -80 ºC. Urinary BPA levels were measured using HPLC-MS. Another cohort of 87 female breast cancer patients was also studied. The female breast cancer patients were recruited at the American University of Beirut Medical Center (AUBMC) between 2012 and 2013 and they signed an informed consent. Their peripheral blood was collected and stored at -80 ºC. Quantitative real-time polymerase chain reaction (PCR) was used to measure the relative telomere length as described by Cawthon's method 2002.

Results: Urinary BPA outliers were calculated using a mathematical formula and there were 39 outliers excluded from the analysis after which the mean ± SD urinary BPA was 2.75 ± 1.6µg/L in the whole cohort after removing outliers and the mean RTL ± SD was 1.42 ±

0.85. Urinary BPA levels and RTL were categorized into tertiles. Univariate analysis showed a statistically significant inverse association between urinary BPA levels and relative telomere length in females only (P= 0.043). After adjusting for urinary creatinine, age, BMI, and postmenopausal status this positive association remained statistically significant (P= 0.067). The same was done after adjusting urinary BPA levels for urinary creatinine and similar results were obtained. RTL in peripheral blood of breast cancer patients was statistically significantly shorter from that of non-breast cancer female controls (mean RTL ± SD: 0.41 ± 0.1 vs. mean RTL ± SD of 1.42 ± 0.76, P= 0.000).After adjustment, the association between relative telomere length and breast cancer risk remained statistically significant (P= 0.00).

Conclusion: This is the first study showing the association between urinary BPA levels and RTL in peripheral blood of Lebanese individuals and also showing the association between RTL and breast cancer risk. We found that high BPA levels were associated with short relative telomere length in peripheral blood of the female subcohort, and at the same time short telomere length is associated with increased breast cancer risk. BPA may be a potential risk factor of breast cancer that needs further study.

CONTENTS

ILLUSTRATIONS

TABLES

CHAPTER I

INTRODUCTION

A. Breast cancer

1. Definition and types

The female breast is a tissue overlaying the pectoral muscle of the chest. It is composed of milk-producing glands called lobules. Milk is transported through a network of tubes called ducts. Breast also contains a fatty and fibrous connective tissue that supports it. In addition, there are blood vessels, lymphatic nodes and vessels for the nourishment and immunity of the breast. Cancer can affect the lobules (Lobular carcinoma) or ducts (ductal carcinoma) of breast (Vorherr, 1974).

Breast carcinoma is a type of cancer that affects the epithelial cells in the glands or cells lining the ducts in the breast. Adenocarcinoma is a type of cancer that affects epithelial cells in the glands, and sarcoma is a type of cancer emanating from connective tissues, fat and muscles. Lobular and ductal carcinomas can either be in-situ whereby cancer does not spread to other tissues; however they can progress into invasive carcinoma whereby cancer cells spread into other tissues through the lymphatic and blood vessels. Most breast cancer invasive cases are invasive ductal carcinoma (8 out of 10), and to a lesser extent invasive lobular carcinoma (1in 10). There are other kinds of breast cancer that are not that common such as Paget's disease, inflammatory breast cancer, angiosarcoma, and phyllodes tumor (CDC, 2014; Vanitha et al., 2014).

There are different breast cancer subtypes depending on the expression of different receptors: estrogen receptor (ER), progesterone receptor (PR) or human epidermal growth factor receptor 2 (Her2). Breast cancer tissues that have positive ER and PR expression have a better prognosis and response to hormonal treatment. On the other hand, women with negative ER and PR breast cancer have a 2-fold increased risk of death relative to positive ER and PR breast cancer (Dunnwald, Rossing, & Li, 2007).

Breast cancer is graded according to the resemblance of a breast cancer tissue to a normal breast tissue, whereby grade 1 means that the tumor is well differentiated and it does resemble normal breast tissue, grade 2 means that cancer cells do not resemble normal ones and they are growing faster and grade 3 means that cancer cells look abnormal and they grow and spread aggressively.

Breast cancer is classified into different stages that help in prognosis and treatment options. The TNM (T: tumor size, N: lymph node involvement, M: metastasis) staging system is the most implemented and it was described by the American Joint Committee on Cancer (AJCC). T reflects the tumor size and it ranges from 0 to 4 cm, the higher the T, the bigger the tumor. N ranges from 0 to 3 and increasing N means increasing in the number of lymph nodes affected by the cancer. M can be either 1 or 0, which means that the breast cancer has or has not metastasized respectively. After grouping T, N, and M we get different stages ranging from I (least advanced) till IV (most advanced) while stage 0 is a non-invasive breast cancer. As breast cancer becomes more advanced, the 5-year survival rate decreases from 100 % to 22 % in stage 0 and stage IV respectively (Howlader et al., 2010).

2. Epidemiology

a. Worldwide

Breast cancer is the most common cancer among women in developed and developing countries with around 1.7 million cases diagnosed in 2012. Around 22% of worldwide cancer cases are breast cancer in women (Ferlay et al., 2014). Breast cancer is the second cause of deaths among women cancer deaths (Vanitha et al., 2014). In 2016, it is expected that there will be 246,660 women diagnosed with invasive breast cancer and additional 61,000 new cases of in-situ breast cancer cases. There is an estimated death of 40,450 women with breast cancer in 2016. Form 1989 till 2012, there was a decline in the breast cancer death rates by 36% due to early detection and advanced treatment (American Cancer Society, 2016). During 2004-2008, the median age of women being diagnosed with breast cancer was 61 years of age. Women between 20-24 years old had the lowest breast cancer incidence of 1.5 cases per 100,000 women, whereas the highest incidence of breast cancer was in women between 75-79 years old with 421.3 cases per 100,000 women (American Cancer Society, 2012; Howlader et al., 2010). In low-income countries, the breast cancer survival rate is below 40% due to poorer early detection and suboptimal treatment facilities (Coleman et al., 2008).

b. In Lebanon

Among all cancers affecting females, 38.2% were breast cancer in 2004 with a median age at diagnosis of 52.5 years. According to the National Cancer Registry in 2003 in Lebanon, 76 new cases per 100,000 women are emerging annually. In 2008, there were

95.7 new cases per 100,000 women and these numbers are expected to continue on rising (Shamseddine et al., 2014). In the cancer country profile for Lebanon compiled by World Health Organization in 2014 (WHO, 2014), most cancer deaths among women were due to breast cancer with a percentage of 26.2%. The highest number of cancer incidences in Lebanon among women is for breast cancer with 1,934 new cases in 2014.

3. Detection

Though the guidelines have recently changed, according to the previous American Cancer Society Guidelines for the Early Detection of Breast Cancer in average-risk, asymptomatic women between 20-39 years of age are recommended to have breast self-examination or clinical breast examination every 3 years. Moreover, women who are 40 years of age and older should undergo annual clinical breast examination and annual mammogram. The doctor can tell if there are any changes in the breast in regards to shape and texture, and observes any abnormal color or redness of skin breast. This can also be done by the woman herself on a regular basis (DeSantis et al., 2011)

Mammogram is an x-ray procedure for early breast cancer detection. Although early detection by mammogram can lead to less aggressive surgeries and treatment, it has some limitations such as the occurrence of false negative and false positive results. In addition to that, some women are worried about the radiation they get exposed to during screening. Yet, mammography is the most effective tool for early breast cancer detection. Another option is the magnetic resonance imaging (MRI) that is a costly technique for

screening, though it gives detailed images of the breast using magnetic field. MRI goes along with mammography but does not replace it (DeSantis et al., 2011).

4. Risk factors

Many factors dispose women tothe risk of getting breast cancer. Age is the most important risk factor after being a female. Most of these factors are related to hormones: prolonged endogenous estrogen exposure due to early menarche<12 years of age, late menopause>55 years of age, or giving first birth at late age(Kelsey et al., 2001). Moreover, exogenous estrogen intake such as hormonal replacement therapy andoral contraceptives use increases the risk of getting breast cancer(Collaborative Group on Hormonal Factors in Breast Cancer, 1996). Breast feeding for at least one year is recognized as a protective factor,whereas short breastfeeding duration is a risk factor for breast cancer(American Cancer Society, 2016).It was shown that African women who didnot breastfeed had higher incidences of ER^-/PR^- breast cancer (Palmer et al., 2011).

Lifestyle is one of the most important modifiable risk factor. For instance, in low and middle income countries, 18% of breast cancer cases are due to lifestyle patterns (Danaei et al.). Obesity or being overweight after age of 18were associated with higher risk of post-menopausal breast cancer(American Cancer Society, 2016; Lacey et al., 2009), yet not many articles have shown a significant relationship between diet and breast cancer risk. Nevertheless, one article showed that high fat intake during adolescence increases the risk for pre-menopausal breast cancer (Linos et al., 2010). In addition, heavy smoking is a risk

for breast cancer (American Cancer Society, 2016). Similarly for alcohol intake, whereby two drinks of alcohol a day were shown to increase the risk for breast cancer by 21%; this could be because alcohol can increase estrogen levels (Singletary & Gapstur, 2001).

Among the non-modifiable risk factors is race whereby in the USA, black women were shown to have more aggressive breast cancer cases and triple negative breast tumors compared to white women (Dunn et al., 2010). White non-Hispanic women were found to have higher breast cancer incidences for most ages compared to African American women (DeSantis et al., 2011). Additional non-modifiable risk factors include genetic mutations in *BRCA1* and *BRCA2* responsible for production of proteins that halt breast cell abnormal growth. *BRCA1* mutation may lead to a 44-78 % risk of getting breast cancer by age of 70, while a mutation in *BRCA2* causes a 31-56 % risk of getting breast cancer (Antoniou et al., 2003). Other genes can predispose women to breast cancer if mutated such as *Tp53*, *CHEK2*, *PTEN*, *CDH1*, and *STK11* (Vanitha et al., 2014). Moreover, having a first degree family history of breast cancer doubles the risk of breast cancer (Lacey et al., 2009).

Most contributing risk factors in Lebanon are obesity and lack of physical activity with percentages of 35.7 and 34.2% respectively. Current tobacco use also poses an important risk factor among women with a percentage of 21.9 % and only 0.8% for alcohol consumption (WHO, 2014). Moreover in the Lebanese society, there is high intake of hormonal replacement that may pose an additional breast cancer risk (Lakkis et al., 2010).

5. Environmental toxins

Estrogen itself is a risk factor of breast cancer, and many studies have shown its carcinogenic effect in in-vitro studies. MCF-10F, human breast epithelial cells, that are ERα-negative and ERβ-positive, were treated with different concentrations of 17 β-estradiol, after which they lost their ability to form normal ductules. In addition to that, they formed a solid mass of cells, they became invasive, and when injected into an animal model they turned into a poorly differentiated adenocarcinomas (Fernandez & Russo, 2010). In another study, high levels of estrogen were associated with increased breast cancer risk in postmenopausal women, especially those with high BMI since adipose tissues contain aromatase enzyme that converts androgen into estrogen (Endogenous Hormones and Breast Cancer Collaborative Group, 2011).

Many articles found an effect of some chemical compounds that are similar to estrogen, on the progression or initiation of breast cancer. These chemicals are known as disruptive endocrine chemicals (DEC) such as: diethylstilbestrol (DES), organ chlorine pesticides (OCP), polychlorinated biphenyls (PCB).

DES was used for several years as an oral contraceptive until it was banned from the market because of its association with vaginal and cervical cancer cases in women who were exposed to DES in their utero life. It was shown that women who took DES during their pregnancies had a higher relative risk of breast cancer (Greenberg et al., 1984)

Some organochlorine compounds that are used as pesticides have estrogen-like activity. Dichlorodiphenyltrichloroethane (DDT), a known pesticide, was banned due to its

strong association with breast cancer risk. In a large cohort, DDT levels were directly correlated with breast cancer risk in a dose dependent manner, and the breast cancer risk was significantly stronger in women who were exposed to DDT before age of 15 when compared to women exposed after age of 15 due to the pre-pubertal and pubertal development of mammary glands that take place in childhood and adolescence (Cohn et al., 2002). These results shed light on the importance of time of exposure; in fact, other case-control studies on DDT failed to show this correlation between DDT and breast cancer risk because DDT levels were measured at the time of diagnosis of breast cancer (Maffini et al., 2006). DDT also showed a shift in estradiol metabolism towards an increased production of 16a-OHE, an estrogen agonist, and decreased transformation of estradiol into OHE1, which is an anti-estrogen. The increase in 16a-OHE/OHE1 ratio was associated with an increase in proliferation and unscheduled DNA synthesis in MCF-7 cells (Bradlow et al. 1995). Other organochloride compounds, including coumestrol (COU), p-tert-octylphenol (OCT) and o,p'-DDT (dichlorodiphenyltrichloroethane), were studied on MCF-7 cell lines. It was shown that these estrogen-like compounds stimulate MCF-7 cells proliferation rate and reduce their apoptotic rate. Moreover, COU and DDT increased the expression of mRNA of progesterone receptor in these cells (Diel et al., 2002).

Polychlorinated biphenyls (PCB) are carcinogenic compounds that were used extensively in the industrial field and they were banned from different countries. Women with breast cancer had high levels of PCBs detected in their mammary adipose tissues (Falck et al., 1992).

Note that many of the environmental toxigens may be activated or deactivated by drug metabolizing enzymes (DMEs), yet in a study done on Lebanese individuals, no association was found between different genetic polymorphisms of DMEs *CYP2E1*, *GST*, and *NAT2* and breast cancer risk (Zgheib et al., 2013).

6. Biomarkers

Different attempts were made in order to discover new breast cancer biomarkers that help in the early diagnosis, and direct the healthcare team in breast cancer treatment. Estrogen receptor has been the most important biomarker since it can be targeted by endocrine therapy (Weigel & Dowsett, 2010). The progesterone receptor, in most cases, is expressed along with the estrogen receptor. Less than one percent of all breast cancers have a positive progesterone receptor along with negative estrogen receptor statuses. It was shown that progesterone receptor expression along with estrogen receptor result in better therapeutic outcomes when breast cancer is treated with an anti-estrogen (Elledge et al., 2000). Her2 is also an important biomarker of breast cancer. Breast cancer patients with overexpressed Her2 have shorter overall survival and are more likely to get a relapse after treatment (Weigel & Dowsett, 2010).

Other biomarkers are currently under study; these include: ERβ, Ki67 and cyclin D1. ERβ mediates different, sometimes opposite, effects from ERα and it was shown to be down-regulated in breast cancer tissues compared to normal breast tissues (Roger et al., 2001). Ki67 is a nuclear non-histone protein that is expressed by highly proliferating cells,

and it was shown that positive Ki67 levels are associated with shorter overall survival in breast cancer patients (Stuart-Harris et al., 2008). Cyclin D1 acts as a positive regulator of the cell cycle and was found to be overexpressed in more than 50% of breast cancer cases at the transcriptional and translational levels. It was shown that cyclin D1 overexpression is a negative predictor of anti-estrogen outcome in postmenopausal breast cancer patients (Stendahl et al., 2004). Further search for breast cancer biomarkers is needed for better screening, diagnosis and prognosis.

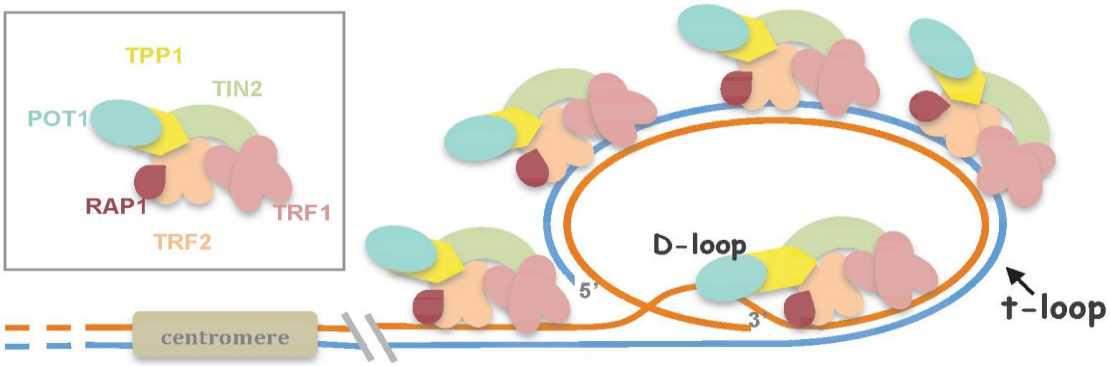
B. Telomere

1. Structure

Telomere is composed of repetitive sequences of six nucleotides (TTAGGG) that are found at the ends of linear eukaryotic chromosomes with a size ranging between 5 to 15 kbp in human cells (Samassekou et al., 2010). Telomere is double stranded and has a long 3’ end that is rich in guanosine facing a 5’ end that is rich in cytosine that always ends with ATC sequence. The 3’ G-strand overhangs and invades the adjacent double strand telomere repeats to form a t-loop in humans, and then it forms base pairs with the facing 5’ C strand forming another loop known as the d-loop, or the displacement loop. Telomeres are bounded to telomere-binding proteins also known as human shelterin. Human shelterin is composed of six proteins, three of which are highly specific: telomere repeat binding factors 1 and 2 (TRF1) and(TRF2) that bind to the double stranded telomeric part, and the protection of telomeres 1 (POT1) that binds to the d-loop and the t-loop. The three other

proteins are repressor/activator protein 1 (RAP1), TRF1-interacting nuclear protein 2(TIN2) and TIN2-interacting protein 1 (TPP1) (Palm & de Lange, 2008) (**Figure 1**).

Figure 1. Telomere structure



Telomere structure capping the ends of linear eukaryotic chromosomes along with human shelterin. Human shelterin is composed of TRF1, TRF2, POT1, RAP1, TIN2, and TPP1. Source: Boutou et al., 2013.

2. Telomerase structure and functions

Telomerase is the enzyme that compensates for telomere loss, and it is detected in 90% of human cancer types (Kim, 1994). It is a ribonucleoprotein composed of a catalytic telomerase protein which is the human telomere reverse transcriptase (hTERT), and the functional template which is the telomerase RNA (TR) of sequence 5' -CUAACCCUAAC-3' complementary to the telomeric repeat (Feng, 1995; Lingner et al., 1997). Telomerase activity is repressed after birth in human somatic cells. Somatic cells were shown to express hTR in certain levels but they have very low levels of hTERT. Telomerase activity is

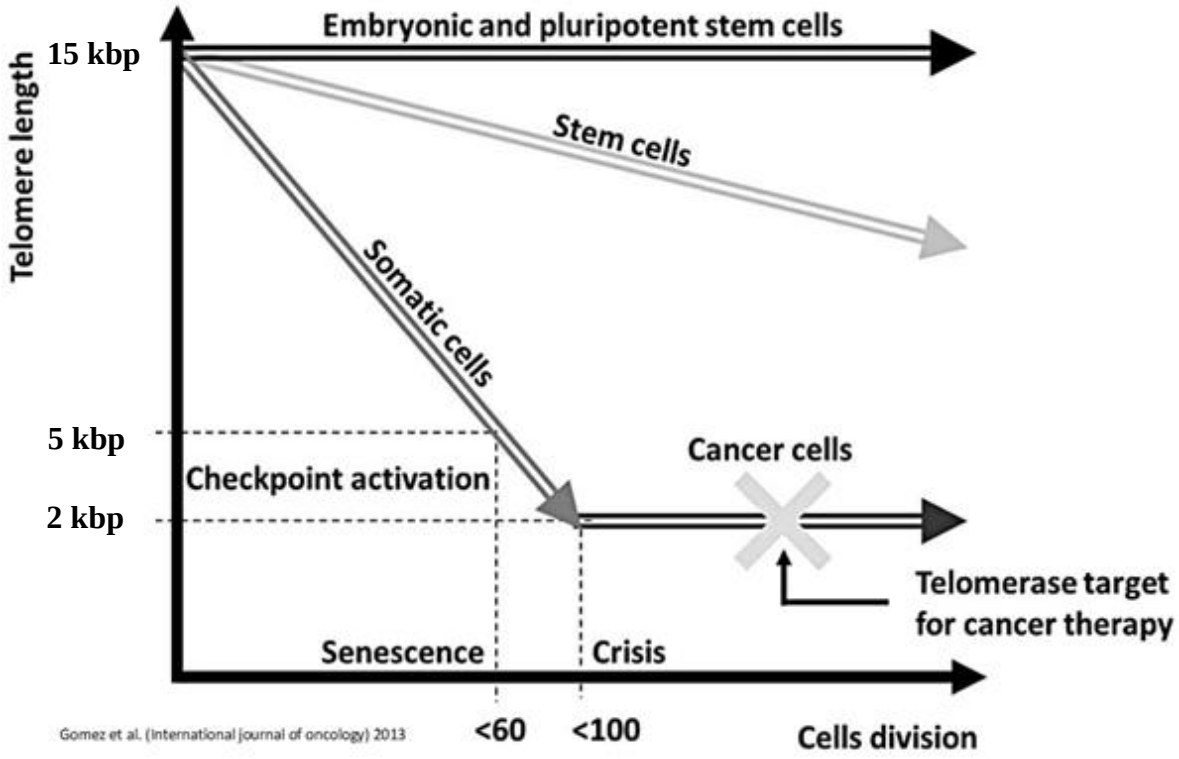
maintained in highly proliferating cells such as keratinocytes and activated lymphocytes (Masutomi et al., 2003; Harley et al., 1990b), germ cells, some stem cells such as hematopoietic progenitor cells or embryonic stem cells (Hiyama & Hiyama, 2007) and other cancer cells (Kim, 1994). Telomerase adds telomere repeats during S or G2/M phase, and it preferentially acts on the shortest telomere subset (Teixeira, Arneric, Sperisen, & Lingner, 2004; Marcand, Brevet, Mann, & Gilson, 2000). Telomerase was found to increase the telomere subset of 100 nucleotides by 42-46%, while 300 nucleotide length telomere is accompanied by only 6-8% increase in telomere length(Teixeira et al., 2004). Nevertheless, Zhao et al. 2009 showed that telomerase in human cancer cells acts on elongating telomere subsets at most of the chromosomes, without having preference for shortest telomeres (Zhao et al., 2009). Telomerase has also been reported to have other functions such as nuclease and transferase activities inside eukaryotic cells, and although telomerase is not active in somatic cells, it was found that hTERT protein can be expressed in the nucleus, cytoplasm and mitochondria and it plays an important role in protecting cells during oxidative stress (Rubtsova et al., 2012).

3. Function

Long telomeres along with shelterin are important for the genetic material stability as they are involved in solving two problems: the chromosome end-protection problem and the chromosome end-replication problem. They play a role in the t-loop formation which is very important in protecting the telomere end andthe chromosome ends from DNA repair machinery, hence avoiding chromosome end fusion through non-homologous end joining

and homology directed repair (Palm & de Lange, 2008). The t-loop limits the access of telomerase into the telomeric end, and thus telomerase access can only be achieved during replication process when t-loop unfolds (Griffith, 1999). Moreover, telomeres along with shelterin avoid the loss of coding nucleotides during each DNA replication. For instance, during each eukaryotic DNA replication, the 5' end of the lagging DNA strand cannot be fully replicated and this is known by the end replication problem, due to the fact that DNA replication only takes place from 5' to 3' direction, and DNA polymerases require an RNA primer (Blackburn, 1991). This results in the loss of 50-200 bp from the telomere during each cell division (Mu & Wei, 2002). So with each cell division, telomere length in human somatic cells shortens till it reaches a critical level. Having one or more critically short telomeres, which is the Hayflick limit, causes the cell to become senescent as it enters the mortality stage 1. At this stage, the cell must not undergo any further division, and if it were able to overcome the cell cycle checkpoints such as p53, it would advance into the mortality stage 2 or cell crisis stage. At this stage, some cells are able to up-regulate the expression of hTERT, hence the telomere length can be maintained and the cells become immortalized and highly proliferative with an increase in genomic instabilities (Cong et al., 2002; Shay & Wright, 2011) (**Figure 2**).

Figure 2. Telomerase and telomere length in different cell



Telomere length as a function of cell division in embryonic and pluripotent stem cells, other stem cells, normal cells, and cancer cells. When telomere length reaches 5 kbp, the cell enters senescence, whereby checkpoints are activated to avoid further cell division. With further cell division, the cells enters crisis with a telomere length of 2 kbp and eventually it will become a cancerous cells with activated telomerase activity to stabilize telomere length. Source: Gomez et al., 2013

4. Age, gender and cell type

An inverse relationship was found between age and telomere length in many articles. This is to be expected since with increasing age, more cell divisions take place leading to a shortening in the telomere length. In vitro studies showed that telomere length in human fibroblasts shortens with each passaging (Harley et al., 1990a), and another study including 137 individuals ranging from 0 to 104 years showed a reduction in their telomere

length with increasing age to a certain degree depending on the organ studied. In addition, cells with rapid turnover were found to have shorter telomeres than static cells of cerebral cortex and myocardium that did not change with age due to the presence of telomere maintenance machinery (Takubo et al., 2002). In human stem cells, a negative correlation was found between age and telomere length despite the presence of telomerase activity in these cells (Vaziri et al., 1994), however, in germ cell lines, it was found that telomere length is positively correlated with age, whereby older men had longer telomeres than younger men in their spermatozoa (Kimura et al., 2008). Gender differences did not seem to affect telomere length at birth, yet it was found that in adulthood, men tend to have shorter telomere length than females. This can be explained by the probability that females use the alternative lengthening of telomere pathway (ALT) more than men, and probably sex hormones play a role in it, though it is not yet fully understood (Muller et al., 2009). ALT is a telomerase independent pathway used by some cancer cells such as renal cell carcinoma and some types of breast carcinoma, and also other telomerase positive and negative cells. Cells that use ALT have a heterogeneous telomere length, which can be extremely short or long. In addition, ALT acts through the presence of extrachromosomal telomere repeats (ECTRs), ALT-associated PML (promyelocytic leukemia) bodies (APBs), and homologous recombination of telomere repeats (Reddel, 2003).

5. Lifestyle

Research has shown that telomere may be affected by lifestyle factors such as: smoking, alcohol consumption, diet, obesity, stress and exercise. Smoking was shown to

shorten telomere in a dose-dependent manner (Morla et al., 2006). Smoking produces many reactive oxygen species that generate an oxidative stress on telomeres affecting their length and eventually accelerate aging (Babizhayev et al., 2011). Alcohol was shown to be associated with shorter telomeres in peripheral blood leukocytes, and it was shown that telomere shortening increases with increasing alcohol consumption (Pavanello et al., 2011). Similarly, post-menopausal women who drank more alcohol had shorter telomere length (Song et al., 2013). Moreover, in a large cohort including 4576 individuals, no correlation was found between alcohol consumption and telomere length in peripheral leukocytes (Weischer et al., 2014). Eating food rich in antioxidants such as: legumes, whole grains, fish, and vegetables was shown to be associated with longer RTL (Lee, et al., 2015; Cassidy et al., 2010). Obesity was also shown to affect telomere length, whereby obese women were found to have significantly shorter telomere length when compared to non-obese women (Valdes et al.,). This can also be attributed to the increased reactive oxygen species production in obese people (Furukawa et al., 2004). Stress is also a cause of telomere shortening, whereby women caring for a chronically ill child, which is considered a source of major stress, had shorter telomeres and less telomerase activity in their peripheral mononuclear blood cells compared to women caring for a healthy child. This shortening of telomere length was equivalent to additional 10 years increase in age in women under stress (Epel et al., 2004). In contrast, exercise was shown to reduce telomere shortening probably due to a decrease in oxidative stress. As a matter of fact, telomere measured from blood leukocytes of athletes demonstrated increased telomerase activity, and less shortening in telomere length compared to that of controls with no regular physical activity (Werner et al., 2009).

6. Estrogen, and estrogen-like chemicals

In human endometrium, it was shown that the telomerase activity was highest during proliferative phase of menstrual cycle, whereby estrogen levels are high. Thus through activating the telomerase, telomere length is protected in the glandular epithelial cells of the endometrium that undergoes proliferation regularly, and after menopause, the decrease in estrogen levels cause attenuated telomerase activity in these cells, and hence protecting against tumorigenesis in the endometrium (Bayne et al., 2007).

Other estrogen-like chemicals were associated with telomere length reduction. For instance, it was shown that Noble rats exposed to diethylstilbestrol (DES) exhibited different changes in their mammary glands, whereby cell cycle of epithelial cells was altered along with increased proliferation and reduction in telomere length. MCF-7 cells treated with BPA and DES showed an increase in telomeric associations that reflects fusion of chromosomes at their ends, hence loss of telomeres and increased genomic instabilities (Roy et al., 1997).

7. Diseases associated with telomere

Telomere length alterations were shown to predispose individuals to many diseases such as: genetic, osteoporosis, cardiovascular diseases, and increased risk of cancers (Shammas, 2011) as shown in **Table 1**. One of the most important genetic diseases affecting telomere length is Dyskeratosis congenita. This is a telomere disorder characterized by skin hyperpigmentation, nails dystrophy and oral leukoplakia. Studies

showed that this disease is due to genetic mutations in the hTR gene or the genes coding for the telomerase RNA-associated protein that lead to defects in the telomerase activity and accelerated reduction in telomere length (De La Fuente & Dokal, 2007).

Shorter telomere length in peripheral leukocytes was associated with a decrease in bone density and osteoporosis in women (Valdes et al., 2007). In addition to that, peripheral leukocytes telomere length was negatively associated with the severity of CHF and atherosclerotic disease progression (van der Harst et al., 2007). In another study of coronary heart disease, patients with short telomere length in their peripheral white blood cells had a 3-fold increase in risk of premature myocardial infarction (Brouilette et al., 2003). Both CHF and atherosclerosis increase oxidative stress and inflammation that affect telomere length negatively. Similar to that, diabetic patients were found to have shorter peripheral lymphocyte telomere length when compared to healthy patients, and were associated with worse vascular wall conditions and higher HbA1c levels (Dudinskaya et al., 2015). In another study, telomere length measured in monocytes was shown to be associated with type 2 diabetes mellitus and with increased oxidative DNA damage in peripheral blood mononuclear cells (Sampson et al., 2006).

Different types of cancer had shown alterations in their telomere length. For example, an increased bladder cancer risk was associated with short telomere length in blood of men and women (McGrath et al., 2007). Patients with cancers in head and neck, lung, and renal cell carcinoma, had shorter telomeres compared to controls. Moreover, the risk of these cancers increased with further shortening of the telomere length (Wu et al., 2003). In colorectal cancer cell lines, cells with longer RTL exhibited higher sensitivity to

anti-EGFR therapy drug (cetuximab) than those with shorter RTL. Moreover, invasive colorectal cancer in patients showing high RTL was associated with better progression-free survival (Augustine et al., 2015).

Table 1. List of diseases associated with alterations in telomere length

Disease		Outcome	Reference
Genetic	Dyskeratosis congenita	Shortening due to defects in telomerase enzyme	De La Fuente et al. 2007
Skeletal	Osteoporosis	Shortening	Valdes et al. 2007
Cardio-vascular	Chronic heart failure	Shortening with severity of CHF	Van der Harst et al. 2007
	Premature myocardial infarction	Shortening with premature MI.	Brouilette et al. 2003
Metabolic	Diabetes Mellitus type 2	Shortening in T2DM and macro-vascular complications	Dudinskaya et al. 2015
	Diabetes Mellitus type 2	Shortening	Sampson et al. 2006
Cancer	Bladder cancer	Shortening	McGrath et al. 2007
	Colorectal cancer	Longer telomere length associated with better progression free-survival	Augustine et al. 2015
	Lung, head and neck, bladder cancer, renal cell carcinoma	Shortening with increased risk of lung, head and neck, bladder cancer and renal cell carcinoma	Wu et al. 2003

CHF: chronic heart failure, MI: myocardial infarction, T2DM: type 2 Diabetes Mellitus

8. Telomere and breast cancer

Few studies were done to identify the relationship between telomere length and breast cancer risk, and the findings were contradictory as shown in **Table 2**. Some papers found that cells with critically short telomere length may escape cell apoptosis and develop cancer with the help of high telomerase activity, that is responsible for stabilizing the short telomere length (Shen et al., 2009; Radpour et al., 2010; Duggan et al., 2014; Raynaud et al., 2010; Odagiri et al., 1994), while others showed that long telomere length was associated with breast cancer risk and decreased survival. Long telomeres may also contribute to increased ability of cancer cells to proliferate, and hence increased genomic instability (Gramatges et al., 2010; Svenson et al., 2008), yet other studies failed to show any association between telomere length and breast cancer (Barczak et al., 2016a; Zheng et al., 2010; De, I et al., 2009).

Duggan et al. showed that the shortening of telomere length in peripheral blood leukocytes in breast cancer female patients within 30 months from diagnosis was associated with worse disease outcome and increase in breast cancer-specific and all-cause mortality (Duggan et al., 2014).Another study showed that premenopausal women having short telomere length were associated with increased breast cancer risk when compared to women carrying longer telomeres in their peripheral blood (Shen et al., 2009). In a study on normal, pre-neoplastic and neoplastic breast tissues, telomere length was found to be shorter in pre-neoplastic tissues compared to normal ones. Invasive breast carcinoma tissues showed two sets of results: one set of tissues had very short telomere length while the other set had a long telomere length. Moreover, ATM (ataxia telangiectasia mutated)

that is a protein kinase with an important role in DNA damage repair machinery, was found to be activated in 70% of these pre-neoplastic lesions and only 14% of invasive breast carcinomas (Raynaud et al., 2010). Consistent results were shown in another study whereby telomere length was shorter in breast cancer tissues compared to adjacent normal breast tissues, tissues with advanced stages and higher tumor grade were significantly associated with even further shortening in the telomere length (Radpour et al., 2010). Similar results were reported by Odagiri et al., however, the study did not find any association between telomere length and breast carcinoma tumor size, TNM stage, or estrogen and progesterone statuses (Odagiri et al., 1994).

Other articles showed longer telomere length to be associated with increased breast cancer risk. For example, breast cancer patients with or without breast cancer family history or *BRCA* mutation had longer telomere length in their peripheral blood compared to controls. Moreover, higher telomere length was associated with controls having breast cancer family history compared to those with no breast cancer family history (Gramatges et al., 2010). Svenson et al. 2008 showed similar results, whereby long RTL was associated with increased breast cancer risk in women after adjustment for age. Along that, shorter telomere length in blood of breast cancer nodal positive patients had better survival rate from those with longer telomere length (Svenson et al., 2008).

Other studies found no difference in the mean telomere length between breast cancer patients and controls; however, it was shown that short telomere length in peripheral leukocytes was associated with advanced stages of breast cancer (Barczak et al., 2016b). Similarly, Zheng et al. and De vivo et al. showed no association between telomere length

and breast cancer risk (Zheng et al., 2010). However, De vivo et al. showed that shorter RTL was significantly associated with older age and high estrone and estradiol plasma levels, but not with other factors such as BMI, smoking status, and ER/PR status (De vivo et al. 2009).

Table 2. List of association studies between telomere length and breast cancer

Outcome	Tissue or blood	Method	Reference
Short telomere length associated with breast cancer risk	Blood	Q-PCR	Duggan et al. 2014
	Blood	Q-PCR	Shen et al. 2009
	Tissue	FISH	Raynaud et al. 2010
	Tissue	Q-PCR	Radpour et al. 2010
	Tissue	Southern blot	Odagiri et al. 1994
Long telomere length associated with breast cancer risk	Blood	Q-PCR	Gramatges et al. 2008
	Blood	Q-PCR	Svenson et al. 2008
No association between breast cancer risk and telomere length	Blood	Q-PCR	Barczack et al. 2016
	Blood	FISH	Zheng et al. 2010
	Blood	Q-PCR	De vivo et al. 2009

Q-PCR: quantitative polymerase chain reaction, FISH: Fluorescence in-situ hybridization

9. Telomerase and cancer

Telomerase activity has been detected in 85-95% of common cancers such as lung, liver, breast cancer, prostate and colon cancers (Shay, 1997). For instance, telomerase activity was detected in 92% of lung adenocarcinoma, 84 % of hepatocellular carcinoma,

and 75.49% of breast cancer tissues, and these were higher than values in normal adjacent tissues. Moreover, high telomerase activity was significantly associated with poorly differentiated lung adenocarcinomas and hepatocellular carcinoma (Fujiwara et al., 2000; Tahara et al., 1995).

As for breast cancer cell lines, MDA-MB-231 cells, an invasive ERα negative human breast cancer epithelial cell line, showed high specific telomerase activity along with short telomere length, in contrast to MCF-7 cells, non-invasive ERα positive human breast cancer epithelial cell line, that showed low specific telomerase activity along with long telomere length. Moreover, it was shown that high specific telomerase activity did not depend on the hTERT expression levels, but rather on the location of hTERT. When hTERT was found uniformly in the nucleus, high specific telomerase activity was seen compared to lower telomerase activity when hTERT was located in the nucleoli (Ceja-Rangel et al., 2016). In breast cancer tissues, higher telomerase activity was seen with bigger size tumors, greater histologic grade, Her2/neu positive status, and Ki-67 expression which implies that high telomerase activity is associated with advanced and more aggressive breast cancer types. This was also associated with a lower 10 years breast cancer-free and overall survival (Kuli-ç et al., 2016). Other cancer cells that are telomerase negative such as osteosarcoma, renal cell carcinoma, some breast carcinomas, and ovarian carcinoma were found to have their telomere length maintained through the alternative lengthening of telomeres (ALT) pathway (Reddel, 2003).

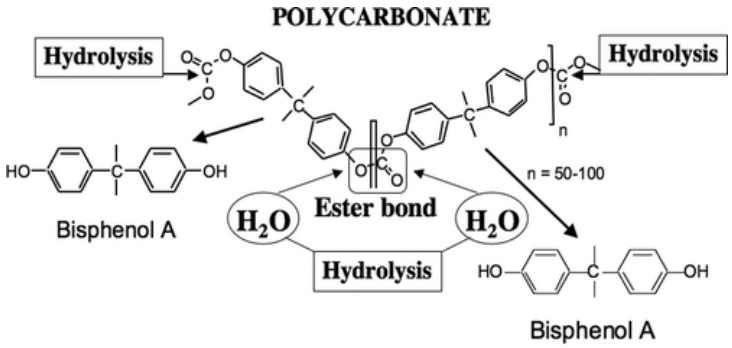
C. BPA (Bisphenol A)

1. Sources

Bisphenol A, also known as 4, 4'-(propane-2,2-diyl)diphenol, is a widely used chemical for the production of polycarbonate plastics and epoxy resins. Polycarbonate plastics have been used extensively since the 1950s in the production of reusable water bottles, baby bottles and food storage containers that can be microwaved and refrigerated(Kawamura et al., 1998; Brede et al., 2003). Epoxy resins are used to coat the inner surfaces of metal cans for preserving food and beverages from bacterial contamination and avoiding metal erosion(Kang et al., 2006), and also for coating drinking water tanks(Bae et al., 2002). In addition, BPA is added to other plastic types such as polyvinyl chloride that forms water pipes, and polyethylene terephthalate which is used in the production of soda and water bottles (Welshons et al., 2006).

Different articles have shown that BPA leaches from plastics upon application of heat, contact with acidic or basic solutions, or frequent brushing and washing of baby feeding bottles and other reusable bottles with hot water or alkaline solutions such as bleach. Also it leaches from different canned food products and microwavable plastics (**Table 3**). In these cases, the ester linkage between BPA molecules hydrolyzes releasing detectable levels of BPA that eventually are ingested (**Figure 3.**) (Kang et al., 2003; Takao et al., 1999). BPA is also solidified by polymerization and used as such in dental fillings that can hence leach BPA. BPA can also be detected in saliva (Sasaki et al., 2005; Zafra et al., 2002).

Figure 3. Hydrolysis of ester linkage Bisphenol-A compounds in polycarbonates.



Source: Welshons et al. 2006

Table 3. Different BPA levels from different products

Sample	BPA level	MOD	LOD	Reference
Plastic baby bottles put in boiling water for 30s	1.2 ng/ml	LC/ED	0.2ng/ml	D'Antuono et al. 2001
Plastic container for microwave	30 ug/g at RT 13.1 mg/g at 40ºC	HPLC/FD	0.04ug/g	Nerin et al. 2003
Canned fish	106 ng/g	GC/MS	1 ng/g	Coa et al. 2011
Beverages	18 ng/g	LC/MS	0.5ng/ml	Horie et al. 1999
Canned pineapples	1.2 ng/g	GC/MS	0.38 ng/g	Cao et al. 2011
Canned Tomato sauce	2.59 ng/g	GC/MS	0.38 ng/g	Cao et al. 2011

MOD: Method of Detection, LOD: Limit of Detection, LC: Liquid Chromatography,ED: Electro-chemical Detector, HPLC: High-Performance Liquid Chromatography, FD: Fluorescence Detection, GS: Gas Chromatography, MS: Mass Spectrometry, LD: Liquid Chromatography, ng: nanogram, ml:milliliters, ug: microgram,g: gram.

2. Environmental exposure

BPA is not only found in plastic products, but it has also been detected in river waters in the USA, Germany, Japan, and The Netherlands (Kang & Kondo, 2006). BPA's

half-life in river water ranges between 3-5 days as it gets degraded under aerobic conditions in the presence of certain strains of bacteria(Kang, Ri, & Kondo, 2004; Kang & Kondo, 2002). BPA has also been detected in seawater with a longer half-life lasting for 30 days (Kang & Kondo, 2005). BPA's presence in water poses a risk when drinking it as well as when bathing or swimming in it, because BPA can cross the skin into the systemic circulation (Demierre et al., 2012). BPA's presence in air is not that critical except for workers using epoxy resin sprays; for instance it was shown that these workers had a higher median urinary BPA level of 1.06 umol/mol creatinine compared to that of a control group of 0.52 umol/mol creatinine (Hanaoka et al., 2002). Soil can also have traces of BPA from humans' wastes, and in this case BPA has a short half-life which is less than 3 days due to chemical bonds formation with soil (Fent et al., 2003; Kawahata et al., 2004).

3. Human exposure

As shown in **Table 4,** there have been several studies documenting BPA levels in different populations, age groups and biological samples. In 2003-2004, the CDC started a National Health and Nutrition Examination Survey (NHANES), whereby it was found that 93% of 2517 urine samples of six years and older human subjects from the USA had detectable levels of BPA ranging from 0.4 ug/L to 149 ug/L and a geometric mean (GM) of 2.6 ng/mL urine. Interestingly, highest total BPA levels were among children between 6 and 11 years old with a GM of 3.4 ng/mL urine. Levels were similar in individuals of 12–19 years of age, 20-59 years of age, and ≥ 60 years of age with GM of 2.8, 2.4, and 2.3 ng/mL urine respectively. Interestingly, females had greater BPA levels than males (Calafat

et al., 2008). Following that, in 2005, 95% of 394 urine samples analyzed had detectable BPA levels with a GM of 1.63 ng/mL urine in American males and 1.12 ng/mL urine in American females (Calafat et al., 2005). More recently, a study done on 922 Chinese individuals' urine samples in 2009 showedthat 58% of males and 44% of females had detectable BPA levels with a GM of 1.41 and 0.58ng/mL urine respectively. It was also shown that higher BPA levels were detected in people above 40 years of age, alcohol drinkers and smokers and those with higher education status (He et al., 2009). These results differed from the CDC study whereby children and females were shown to have highest BPA levels.

BPA levels were also detected in tissues such as maternal blood, fetal cord blood, amniotic fluid, placenta, and breast milk. For instance, BPA levels were detected in the maternal blood of 40 American women at delivery with a mean of 5.9 ± 0.94 ng/mL that poses a risk of fetal exposure to BPA, especially that BPA's clearance is slower in the fetus due to immature liver (Padmanabhan et al., 2008). Another study, including 300 maternal blood and fetal umbilical cord blood samples of Korean women at delivery, showed a mean maternal blood BPA level of 9.04 ± 0.81 ng/mL versus 1.13 ± 0.08 ng/mL in fetal cord blood, and there was a positive correlation between the maternal and fetal blood BPA levels (Lee et al., 2008). Similarly, a study on Japanese women showed higher levels of BPA in fetal serum with a mean of 2.2 ± 0.318 ng/mL compared to 1.4 ± 0.148 ng/mL in maternal serum taken at late pregnancy. In the same study, BPA levels were detected in amniotic fluid taken early between 15-18 weeks of pregnancy and at full term pregnancy: it was found that higher levels of BPA were present in amniotic fluid at early pregnancy with a

mean of 8.3 ± 1.573 ng/mL versus 1.1 ± 0.162 ng/mL in amniotic fluid taken at full term. It is noteworthy that BPA levels taken from amniotic fluid at early pregnancy were 8-folds higher than those from maternal plasma taken at the same time. Higher BPA levels found at the beginning of pregnancy are due to maternal plasma crossing the placenta membrane and reaching the fetus that does not have a mature liver yet, and that is why BPA accumulates. As pregnancy reaches its full term, the fetus has a relatively mature liver that can metabolize BPA to a certain extent. In addition, the fetal urine carrying BPA becomes diluted with the maternal plasma. That is why results did not show any significant correlation between mother and fetus BPA serum levels which might be explained by the presence of partial metabolism of BPA in fetus (Ikezuki et al., 2002). BPA was also detected in placenta of 37 German women at delivery with a mean of 11.2 ± 1.512 ng/g (Schonfelder et al., 2002).

A study was done to detect BPA levels in human colostrum to understand the extent of newborn exposure to BPA through breastfeeding. High levels of BPA were measured in 101 human colostrum samples with a mean BPA level of 3.41 ± 0.13 ng/mL. These results were compared to BPA levels in maternal sera of the same healthy women, and it was shown that BPA levels were higher in human colostrum (Kuruto-Niwa et al., 2007). In human breast milk, BPA levels were detected with a mean of 0.61 ± 0.2 ng/mL (Sun et al., 2004), and in another study including breast milk of 20 American women, mean BPA level was 1.9 ng/mL in breast milk (Ye et al., 2006).

4. Estimated daily intake

Toxicological studies on Sprague-Dawley rats showed that the no-observed-adverse-effect-level (NOAEL) is 5 mg/kg/day, where by the critical effects were changes in the body and organ weights of adult and offspring rats (Tyl et al., 2002). After that, NOAEL was divided by 100 for intra-species and inter-species differences to get a tolerable daily intake dose (TDI) of 0.05 mg/kg/day. Recently, the EFSA had refined the results. A new dose called the benchmark dose which is the lowest dose causing an enlargement in the kidneys of mice, was used to calculate the human equivalent dose. It was found that benchmark dose is 8960 ug/kg/day and the human equivalent dose is 690 ug/kg/day. From the human equivalent dose a temporary TDI (t-TDI) was calculated after accounting for data uncertainties and differences in species and individuals; the end result was a t-TDI of 4 ug/kg/day (EFSA, 2015).

The European food safety authority (EFSA) estimated that BPA dietary intake was 0.875 ug/kg/day in toddlers and infants, 1.449 ug/kg/day in adolescents, and 0.388 ug/kg/day in adult women and men (EFSA, 2015).

In addition, the US FDA estimated a human daily BPA exposure of 0.2-0.5 ug/kg/day (mean-90^{th} percentile) for US population 2 years of age and older (FDA, 2014). The NHANES in 2003-2004 found that BPA daily intake from all routes in US population is 0.051 ug/kg/day (Lakind et al., 2008).

Table 4. Several studies documenting BPA levels in different populations in different tissues

Reference	Number of participants	Country	LOD ng/ml	Tissue Type	Mean levels of BPA in ng/mL
Sasaki et al. 2005	21 dental patients	Japan	Not mentioned	Saliva	32.1 ± 16.27 (immediately after restoration of one dental composite)
Zafra et al. 2002	8 dental patients	Spain	3	Saliva	26.125 (one hour after dental composite restoration)
Calafat et al. 2005	394 adults	US	0.1	Urine	GM= 1.33 Adj. GM= 1.36 µg/g
Calafat et al. 2008	2517 (≥ 6 years of age)	US	0.4	Urine	GM= 2.6
He et al. 2009	992 adults	China	0.31 (urine) 0.39 (blood)	Urine Blood	GM= 0.87, adj. GM= 0.38 ug/g GM= 0.18
Padmanabhan et al. 2008	40 women at delivery	USA	0.5	Maternal blood	5.9 ± 0.94
Lee et al. 2008	300 women at delivery	Korea	0.625	Maternal and fetal blood	Maternal blood: 9.04 ± 0.81 Fetal blood: 1.13 ± 0.08
Ikezuki et al. 2002	37 Healthy women (early and late pregnancy) 32 fetal cord serum	Japan	0.3	Maternal and Fetal serum Amniotic fluid	Early pregnancy: 1.5 ± 0.197 Late maternal serum: 1.4 ± 0.148 Fetal (cord) serum: 2.2 ± 0.318 Early amniotic fluid: 8.3 ± 1.573 Late amniotic fluid: 1.4 ± 0.148
Schönfelder et al. 2002	37 women at delivery	Germany	0.01	Maternal and fetal serum Placenta	Maternal serum: 2.9 ± 0.411 Fetal serum: 4.4 ± 0.641 Placenta: 11.2 ± 1.512 ng/g
Kuruto-Niwa et al. 2007	101 women 3 days after delivery	Japan	0.3	Human colostrum	3.41 ± 0.013
Sun et al. 2004	23 women	Japan	0.11	Breast milk	0.61 ± 0.042
Ye et al. 2006	20 women	USA	0.28	Breast milk	Total BPA: 1.9

LOD: limit of detection, GM: geometric mean, adj. GM: creatinine adjusted BPA geometric mean, ng: nanogram, ml: milliliter, ug: microgram, and g: gram

5. BPA and Estrogen

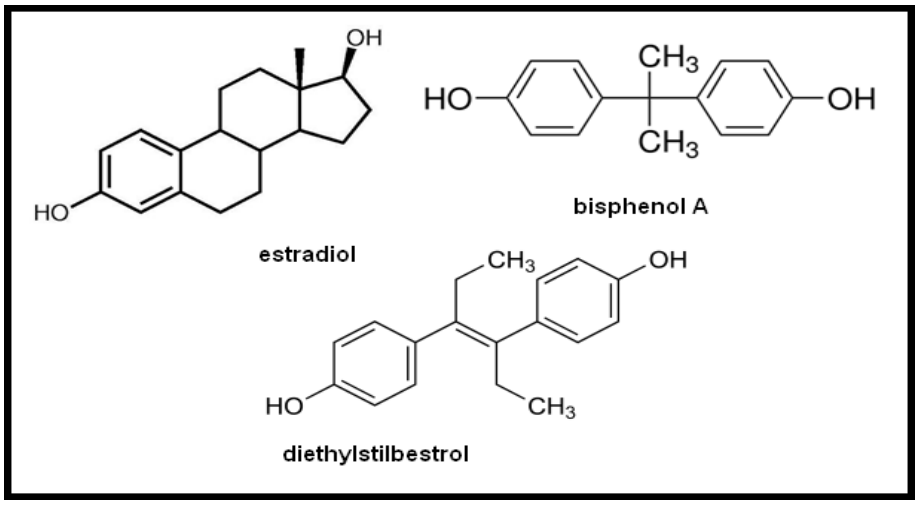
BPA was synthesized by A. P. Dianin in 1891 and was studied in order to be used commercially as a synthetic estrogen in the 1930s by Edward Charles Dodds. It was confirmed that BPA has an estrogenic activity, but another synthetic estrogen which is diethylstilbestrol (DES) was found to have a more potent estrogenic activity than BPA, and that is why BPA development was dropped in favor of DES. In the 1950s, several chemists around the world started using BPA to produce polycarbonate and epoxy resins, and it was extensively used in the industries for the production of plastics, electronics, CDs, DVDs, electrical appliances, automobiles, and dental sealants. In the 1971, DES was banned after it was prescribed to many women with pregnancy related problems because it was found to be linked to vaginal and cervical cancers in female offspring of mothers who took DES. It was also banned from being used in animals to increase their meat production in the 1979 by the US FDA (Rubin, 2011; Vogel, 2009).

There has also been a growing concern about BPA due to many reasons. First, BPA is ubiquitous in nature and it is capable of entering the human body through several routes (dermal, oral, and inhalation). Second, DES and BPA are both synthetic estrogens with similar structure (shown in **Figure 4.**), and having found that DES is linked to cancer raises a question as to whether BPA has a similar link (Rubin, 2011; Vogel, 2009). The American Plastic Council (APC) funded a report by Grey et al. in 2004, and showed that low doses of BPA have no evident adverse effects. However in the following year, another article was issued by von Saal and Hughes (who is a member of the APC) showing that research was failing to unravel side effects of BPA due to the limited funding and

inappropriate animal models used. For instance, Sprague-Dawley rats that are used in BPA assessment studies are insensitive to low levels of BPA, and thus they do not resemble the human body (Maffini et al., 2006).

In light of these study limitations and knowing that BPA is an estrogen-like compound, the Canadian government classified BPA as "toxic" and it has been seeking a limited ban on BPA use. However, US FDA and the EFSA considered BPA as a safe product since the estimated levels of BPA were below the tolerable daily intake (TDI) (EFSA, 2015).

Figure 4. Structure of estradiol, BPA, and DES



6. Pharmacokinetics

BPA is completely and rapidly absorbed in the gastrointestinal tract of humans after ingestion, as it is lipophilic with an octanol-water partition coefficient ($K_{o/w}$) ranging

between 2.2 and 3.82 (Edginton & Ritter, 2009). Bisphenol A undergoes rapid and extensive first pass metabolism in the human liver and small intestine through the glucuronidation pathway leading to BPA glucuronide byUridine 5'-diphospho-glucuronosyltransferase (UGT2b1) following first order kinetics, and to a lesser extent (around 7% of oral BPA) through sulfation in liver (Matsumoto et al., 2002; Kurebayashi et al., 2010). Orally administered BPA of 100 ug/kg/day was given to men and women and it was found that the maximum total serum BPA level was 390 ng/ml after 1.1 hours of oral intake, and maximum unconjugated serum BPA level was 1.5 ng/ml after 1.3 hours of oral intake, and it started to appear in blood after 20 min of oral intake (Thayer et al., 2015). UGT2B1 is expressed in kidneys and intestines but to a little extent compared to liver, whereas it is absent in the lungs (Trdan Lusin et al., 2012).

BPA glucuronide and BPA sulfate lack estrogenic activity.In rodents, it was shown that conjugated BPA is de-conjugated in the lower intestine, and this is relevant in humans in general and fetuses in particular as, in the human body, glucuronidases are produced during infancy in the digestive tract, which means that conjugated BPA may be de-conjugated in the intestines at early age. Moreover, fetuses and neonates are expected to have low levels of UGT2B1 as their liver didn’t reach full maturation yet (Vandenberg et al., 2009). Note that estrone sulfatases, present in different tissues, also de-conjugate BPA sulfate into free BPA. Glucoronidated BPA is mainly eliminated in the urine and feces, and although there is no direct evidence in humans yet, BPA may be subjected to entero-hepatic circulation (Yang et al., 2015).

BPA was also shown to be metabolized by cytochrome P450 2 (CYP2) family members: CYP2C9, CYP2C19 and CYP2C18 with CYP2C9 having the highest affinity to BPA. BPA was also shown to competitively inhibit progesterone metabolism by binding to CYP2C19 (NIWA et al., 2001).

BPA binds to albumin, though with low affinity. It also binds to sex hormone binding globulin (SHBG) in human plasma (Teeguarden et al., 2005). BPA has been detected in breast milk, fetal plasma, and placentain many human studies (Vandenberg et al., 2010).

7. Pharmacodynamics

BPA is a well-known environmental estrogen and an endocrine disruptor. It acts on different types of receptors as shown in **Figure 5.** It binds to different ligand binding domains on estrogen receptor (Gould et al., 1998) with a 10-fold higher affinity to ERβ compared to ERα. Structure activity relationship shows that the 4-OH group, the two benzene rings, and the hydrophobic propane are important for the BPA estrogenic activity. Although BPA is an estrogen-like compound, it has a very low potency to ER with an EC_{50}= 2-7 $\times 10^{-7}$ M compared to that of estradiol EC_{50}= 1-6$\times 10^{-13}$M (Andersen et al., 1999), yet it can also activate many different transcriptional regulators affecting the cellular responses at doses lower than those needed to bind to ER.

When it acts on ERβ, BPA activates TIF2 co-activator, and activates co-activator-1a through either ERα or ERβ in HeLa cells (Routledge et al., 2000). Moreover, and

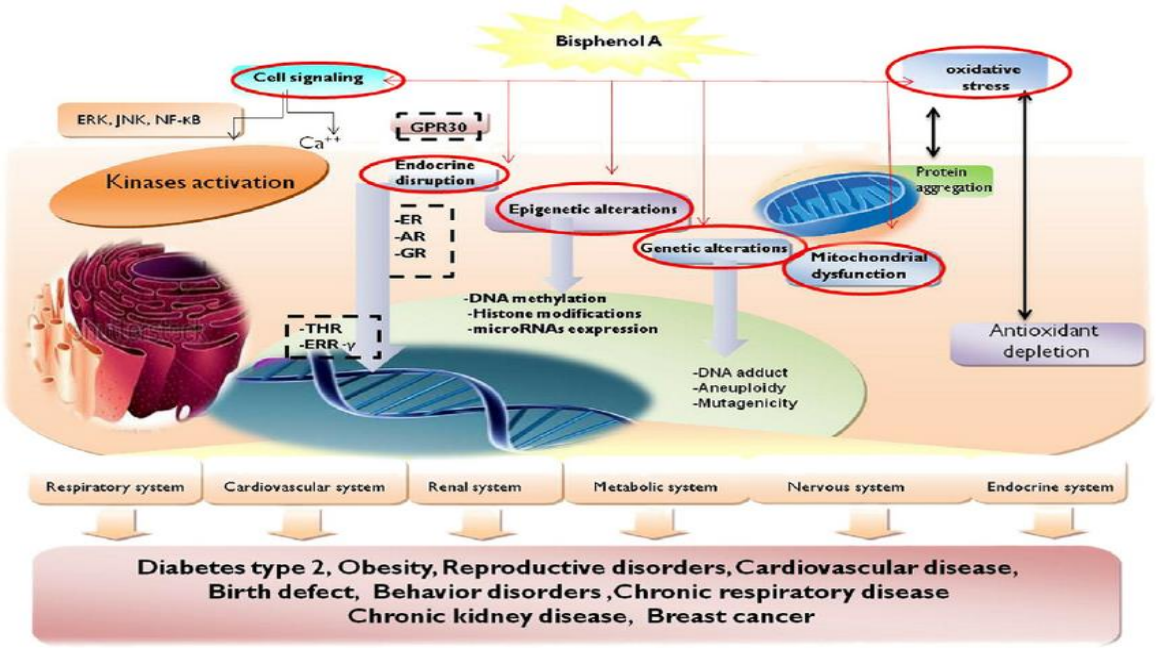
similarly to estrogen, BPA was found to have non-genomic effects(Wade et al., 2001) through binding to membrane estrogen receptor (mER) and trans-membrane estrogen receptor known as GPCR30 with a binding affinity of 8-50 times to GPCR30 higher than that to ERα and ERβ (Thomas & Dong, 2006). BPA can induce prolactin release by stimulating calcium influx via L-type channels through binding to mER (Watson et al., 2007).

BPA is an antagonist of estrogen-related receptor γ (EER-γ), a subfamily of orphan nuclear receptor that is important in the development of fetal brain. EER-γ is constitutionally active and by BPA binding to it, it prevents the inverse agonistic effect of some anti-estrogens such as 4-Hydoroxytamoxifen (Takayanagi et al., 2006). BPA was shown to interfere with androgen receptor (AR), whereby it competitively binds to it and inhibits AR nuclear translocation at a concentration of 1-2 uM (Teng et al., 2013). BPA was also found to be a glucocorticoid receptor agonist similarly to dexamethasone. For instance, in vitro studies showed that BPA stimulated adipogenesis through glucorticoid receptor (Sargis et al., 2010), and in in vivo studies on rats, female offspring exposed to BPA prenatally had increased levels of corticosterone under mild stressful conditions when compared to control (Poimenova et al., 2010). Moreover, BPA was shown to inhibit the aryl hydrocarbon receptor (AhR) at5×10^{-5} to 10^{-4} M. AhR, a transcription factor with an AhR nuclear translocator (AhRnt), is hypothesized to regulate the expression of aromatases including CYP19 responsible for the synthesis of estrogen from testosterone, hence disrupting the balance between androgen and estrogen affecting both female and male reproductive systems. Moreover, AhR cross talks with other receptors such as ERs and AR

whereby BPA's antagonistic effect on AhR can lead to many adverse effects (Bonefeld-Jorgensen et al., 2007).

A study showed that BPA inhibits the binding of T_3 to its thyroid receptor and inhibits its transcription through the recruitment of N-CoR, a nuclear receptor co-repressor at the promoter site (Moriyama et al., 2002). Finally, BPA can cause epigenetic alterations especially regarding DNA methylation. BPA was found to induce hypo-methylation at *PDE4D4* gene site which increases the expression of this gene and can be associated with prostate cancer proliferation. Phosphodiesterase type 4 variant 4 (*PDE4D4*) gene codes for the *PDE4D4* enzyme that degrades cAMP and was linked to prostate cancer. (Ho et al., 2006).

Figure 5. Different pharmacological effect of BPA



Source: Rezg et al. 2014 (Environment International)

8. Toxicology of BPA

Studies have been controversial as to whether environmentally relevant BPA exposure poses a risk to human health. Different articles found that at lower doses than TDI, BPA did induce adverse health effects in in-vivo studies on animals. This could be attributed to the wide pharmacological properties of BPA and the different signaling pathways it can stimulate. In addition, and similarly to estrogen, BPA seems to follow the non-monotonic dose response model whereby low levels might exhibit different responses when compared to higher doses (Vandenberg et al., 2009).

9. Adverse effects

BPA was shown to cause many side effects in animal models and association studies in human subjects.

a. Animal studies

Mice exposed to BPA doses of 25 or 250 ng/kg/day showed a change in the anatomy of the mice ovaries, whereby the volume of antral follicles occupying the ovaries increased and that of corpora lutea decreased. This means a decrease in the number of ovulated oocytes in 3-month old mice (Markey et al., 2003). In another study, female juvenile mice exposed to increasing doses of BPA from 15 to 70 ug/kg/day showed an increased risk in aneuploidy in oocytes in a dose dependent matter (Hunt et al., 2003). Using rat models, BPA doses of 0.1-1.2 ug/kg/day exposure during pregnancy and lactation led to having female offspring with increased body weight even during adulthood hence the

risk of obesity, and the results were more striking in females when compared to male offspring. It was also found in the same study that rats exposed to BPA had lower levels of luteinizing hormones in blood compared to controls, and they showed early onsets of puberty (Rubin et al., 2001). BPA was also shown to affect male reproductive system, whereby mice exposed to 2ng/g of BPA in utero life were found to have later on increased prostate size and decreased sperm production (vom Saal et al., 1998). BPA, given at low doses similar to the tolerable daily intake dose 0.05 mg/kg/day, was found to induce oxidative stress and disrupt mitochondrial function in rat livers (Moon et al., 2012). BPA can also affect the immune system; for instance prenatal exposure to BPA caused an increase in the production of a specific antibody when mice were exposed to its relative antigen; this appeared at low doses of 30 ug/kg/day and in a dose dependent manner (Yoshino et al., 2004). BPA can decrease the levels of antioxidants by unknown mechanisms which can be either through inhibiting antioxidant enzymes or increasing reactive oxygen species production leading to enzyme depletion that can happen at low doses of 0.2 ug/kg/day in adult males (Chitra et al., 2003).

b. Human studies

Different human studies had shown the association between BPA and human diseases as shown in **Table 5**. As shown in animal studies, some articles had found an association between BPA and birth defects and developmental disorders. BPA was previously shown to accumulate in the fetus due to its inability to metabolize BPA which may be posing dangerous consequences on the fetal life. Aneuploidy is a known leading cause of miscarriages in humans, and that it was proposed that high BPA levels may be

linked to recurrent miscarriages. For instance, Sugiura-Ogasawara et al. found higher levels of BPA in the blood of women with recurrent miscarriages compared to women who completed their full term pregnancy. Moreover in the same study, tissues of embryos were found to have an abnormal karyotype similar to that caused by high levels of BPA (Sugiura-Ogasawara et al., 2005). Another study found that mothers exposed to BPA delivered newborns with low birth weight, and alterations in leptin and adipokine secretions posing a metabolic risk (Chou et al., 2011).

Other associations of BPA with the reproductive system have been seen as well. For example, male workers with high BPA levels were found to have problems in erection and ejaculation and reduced sexual drive (Li et al., 2010b; Li et al., 2010a). As for women undergoing in vitro fertilization, high BPA levels were associated with a decrease in oocyte retrieval and estrogen peak levels (Mok-Lin et al., 2010).

It was also found that high urinary BPA levels were associated with cardiovascular heart diseases. Since BPA is lipophilic, it accumulates in fatty tissues which is accompanied with slow release of BPA, and also since BPA was linked to increase in inflammatory markers, these may be a potential cause behind the damage to the endothelial cells that precipitate cardiovascular problems (Melzer et al., 2010). Moreover, another study showed that high urinary BPA levels were associated with hypertension, and this was independent of confounding factors as BMI and diabetes (Shankar & Teppala, 2012). BPA serum levels were found to promote echogenicity of intima-media complex and overt plaques, which means a possible role for BPA in atherosclerosis, and hence associated cardiovascular events (Lind & Lind, 2011).

BPA also seems to play a role in endocrine and metabolic diseases. For instance, high urinary BPA levels were associated with high obesity prevalence among adults, whereby BPA was shown to cause insulin resistance and increased lipid production, and this was first assessed in in vitro studies, whereby BPA caused adipogenesis as mentioned earlier, and also inhibition of adiponectin secretion in a dose dependent manner, and thus disrupting the effect of adiponectin on glucose and fatty acid metabolism. Moreover, BPA's antagonistic effect on the thyroid hormone receptor and peroxisome proliferator-activated receptor γ can mediate insulin resistance and obesity (Wang et al., 2011). Interestingly, similar results were found in children and adolescents, whereby high urinary BPA levels were found to be associated with obesity (Trasande et al., 2012). Although Lakind et al found no association between BPA and diabetes (LaKind et al., 2012), many articles reported it. For instance, by acting on ERα, BPA stimulates insulin secretion leading to hyperinsulinemia and insulin resistance (Sun et al., 2014). Similar results were reported by Shankar et al., whereby higher levels of urinary BPA were associated with type 2 diabetes mellitus, and this could be indirectly caused by obesity and insulin resistance that were also associated with high BPA levels (Shankar & Teppala, 2011).

In addition to these, high BPA levels were associated with a decrease in renal function, as patients on hemodialysis had 6-folds higher BPA concentration when compared to patients with chronic kidney disease stage 5 but not put on dialysis yet (Krieter et al., 2013). BPA was also found to be associated with altered liver functions since high BPA levels were associated with abnormal serum liver enzymes levels (γ-glutamyltransferase, alkaline phosphatase, and lactate dehydrogenase), meaning that BPA

may cause liver damage, a finding that needs further assessment and exploration (Lang et al., 2008).

BPA was also linked to respiratory disorders. For example in a cohort that included 568 asthmatic children, it was shown that exposure to high BPA levels was associated with an increase in wheezing and asthma (Donohue et al., 2013). Regarding the immune system, high urinary BPA levels were associated with increased cytomegalovirus antibody levels in adults, which means that the virus is able to survive and replicate in the human body, and that may be why the immune system increases its antibody production to counteract it. BPA was found to disrupt macrophages by altering their adhesion in rats, and that is a possible way for BPA to decrease the immune functions (Rees Clayton et al., 2011). Finally, a study found an association between high BPA levels and increased inflammatory and oxidative stress markers levels in postmenopausal women, but not in premenopausal women or men (Yang et al., 2009).

Table 5. List of studies showing association between BPA levels and diseases

Reference	Source	BPA levels (ng/ml)	Outcome
Sugiura-Ogasawara et al. 2005	Blood	Mean in 45 cases: 2.59±5.2l Mean in 32 controls: 0.77 ± 0.38	High BPA levels associated with recurrent miscarriages
Chou et al., 2011	Blood	GM in 97 maternal blood: 2.5 GM in 97 fetal cord blood: 0.5	BPA associated with low birth weight and alteration in metabolic cytokines.
Li et al, 2010	Urine	Median in 427 male: 53.7 ug/g creatinine IQR: 8.6–558.9	BPA associated with problems in men sexual activity
Mok-Lin et al., 2010	Urine	GM in 84 women: 2.52	BPA associated with decrease in oocyte number
Melzer et al., 2010	Urine	GM in 1493 adults:1.79 95% CI: 1.64-1.96	BPA associated with heart diseases
Shankar et al., 2012	Urine	GM in 1380 subjects: 2.6 95% CI: 2.4–2.9	BPA associated with hypertension
Lind et al., 2011	Serum	Median in 1016 subjects: 3.76 IQR: 2.02-6.52	BPA associated with plaque echogenicity
Wang et al. 2012	Urine	Median in 3390 subjects: 0.81 IQR: 0.47-1.43	BPA associated with obesity and insulin resistance in adults
Trasande et al., 2012	Urine	Median in 2838 subjects: 2.8 ng/ml IQR:1.5-5.6	BPA associated with obesity in children and adolescents
LaKind et al., 2012	Urine	GM: 2.6,95% CI: 2.4–2.9	BPA is not associated with diabetes mellitus or heart diseases
Sun et al., 2014	Urine	In 971 T2DM individuals: median: 2.3, IQR: 1.4-3.8 971 Controls: median: 2.0, IQR: 1.3- 3.5	BPA associated with T2DM and insulin resistance
Shankar et al., 2011	Urine	Mean in 1916 men: 3.97 ± 0.21 Mean in 2051 women: 3.90 ± 0.26	BPA associated with T2DM and obesity
Lang et al., 2008	Urine	Mean in obese individuals: 6.93, 95% CI: 4.39-9.47 Mean in controls: 3.91, 95% CI: 3.34-4.48 (total 1455 subjects)	BPA associated with increased serum level of liver enzymes
Donohue et al., 2013	Urine	Mean in 568 subjects: 6.0 ± 6.5	BPA associated with asthma in children
Clayton et al., 2011	Urine	In 787 CMV + sera individuals: 5.53	BPA negatively affects immune function
Yang et al., 2009	Urine	GM: 0.56 ug/g creatinine in all 485 subjects GM: 0.58 ug/g creatinine in 134 postmenopausal women	BPA associated with oxidative stress in postmenopausal women

GM: geometric mean, IQR: interquartile range, 95%CI: 95% confidence of interval

10. BPA and breast cancer

Different in vitro, in vivo, and human studies were done to test if BPA is involved in breast cancer. In vitro studies using MCF-7 cells treated with BPA have shown an increase in the number of treated MCF-7 cells through slowing down apoptosis rate rather than increasing proliferation (Diel et al., 2002). Moreover, the human breast epithelial MCF-10F cells, treated with BPA concentrations of 10^{-5} and 10^{-6} M formed more solid masses and less tubules compared to control (Fernandez & Russo, 2010).

Different animal models were used to study the effect of BPA on mammary glands as shown in **Table 6**. Using MMTV-erbB2 female mice, it was shown that chronic exposure of low levels of BPA that are similar to human daily intake of BPA caused an increase in the mammary gland tumor burden, and cellular proliferation as well as incidence of metastasis (Jenkins et al. 2011). Another study using pregnant CD-1 mice exposed to low doses of 25 or 250 ng/kg/day BPA, showed that female offspring at day 30 of age had increased number of terminal end ducts, which means an increase in the ductal growth of their mammary glands, which is due to a decrease in rate of apoptosis at terminal buds with no effect on proliferation rate. Moreover, BPA increased estrogen sensitivity of the mammary glands, and increased progesterone receptor expression, but not estrogen receptor expression in ductal epithelial cells (Munoz-de-Toro et al., 2005). In another study, fetal exposure in a rat model of low doses of 2.5 and 25 ug/kg/day of BPA, showed at postnatal day 50 increased rate of intraductal hyperplasias and carcinomas in mammary glands in-situ with increased expression of estrogen receptor α at postnatal day 50 (Murray et al., 2007). Similar results were shown in a different study, whereby a BPA dose of 25

ug/kg/day given to Wistar rats increased hyperplastic ducts and decreased apoptotic index with a slight increase in proliferation (Durando et al., 2007). Another study showed that pregnant and lactating Sprague-Dawley rats exposed to different doses of BPA including 0.25, 2.5, and 25 ug/kg/day led to the presence of pre-neoplastic and neoplastic lesions in mammary glands of their female offspring at postnatal day 90 (Acevedo et al., 2013). Male mice were studied as well, and they were exposed during gestation and lactation to different doses of BPA, and it was shown that low BPA doses of 2.5ug/kg/day increased the branching in the ductal area of mammary glands at a young age (Vandenberg et al., 2013).

Tissues from humans were also studied along with association studies to evaluate whether BPA in humans has similar effects to those seen in cell lines and animal models. In an interesting study, non-malignant contralateral breast tissues were taken from breast cancer female patients through aspiration. These tissues were cultured and treated with a BPA concentration of 10^{-7} M. It was found that BPA induced different gene expressions that were shown to decrease apoptosis and increase cell tolerability to micro-environmental stress without affecting cell proliferation. Moreover, different gene expression due to BPA were shown to be prevalent in tumors with ER negative status, more than 2cm size, and higher histological grade that are all indicators of aggressive breast cancer (Dairkee et al., 2008). In contrast, Yang et al. studied Korean women with breast cancer and the study found that high blood BPA levels were negatively associated with age at first birth, and no association with breast cancer risk (Yang et al., 2008). Finally, it was shown that high serum levels of BPA were associated with increased mammographic breast density in postmenopausal women which is a risk factor of breast cancer (Sprague et al., 2013).

Table 6. BPA doses, exposure route and duration in different studies to study its effect on mammary glands in animal models

References	Animal model	BPA exposure route	BPA doses or concentration	Duration of exposure	Outcome
(Jenkins et al., 2011)	MMTV-erbB2 mice	Drinking water	2.5, 25, 250, or 2,500 ug/l	56 days of age for their lifetime	Increased mammary tumorigenesis and metastases at low doses in females
(Munoz-de-Toro et al., 2005)	CD-1 mice	Alzet osmotic pump	25 or 250 ng/kg/day	Gestational day 9 till postnatal day 4	Increased ductal growth of mammary glands of females
(Murray et al., 2007)	Wistar-Furth rats	Alzet osmotic pump	2.5, 25, 250 or 1000 ug/kg/day	Gestational day 9 of till postnatal day one	Increased ductal hyperplasias and carcinoma in mammary glands of females
(Acevedo et al., 2013)	Sprague-Dawley rats	Alzet osmotic pump	0.25, 2.5, 25, or 250 µg/kg/day	Gestational day 9 to birth or postnatal day 21	Increased preneoplastic and neoplastic lesions in mammary glands of females
(Durando et al., 2007)	Wistar rats	Alzet osmotic pump	250 ug/kg/day	Gestational day 8 till gestational day 23	Increased hyperplasia in mammary glands of females
(Vandenberg et al., 2013)	CD-1 mice	Alzet osmotic pump	0.25, 2.5, 25, or 250 ug/kg/day	Gestational day 9 till postnatal day 16	Increased ductal branching in mammary gland of males

CHAPTER II

RATIONALE OF STUDY AND AIMS

Breast cancer has been studied extensively throughout the years. With increasing number of breast cancer incidences all over the world, there is an increasing urge to find other advanced ways to understand breast cancer etiology. Understanding what is happening at the molecular level paves the way for new methods to help in early detection of breast cancer. Moreover, it provides researchers with new mechanisms to combat breast cancer cells and probably expanding the results to other types of cancers and diseases.

Telomere length was shown to be an important marker of aging. As people get older, telomere length was found to get shorter and this may predispose human beings to different diseases. Telomere length was also found to be altered in breast cancer. Similarly, Bisphenol-A (BPA), which is a known chemical compound that acts similarly to estrogen, is found ubiquitously in our environment. It can enter the human body through different routes (oral, pulmonary, and dermal) and from many different sources. It was shown that BPA induced telomeric associations in MCF-7 breast cancer cell lines (Roy et al., 1998) and thus its association with telomere length in human beings is warranted.

In this study, we hypothesized that high urinary BPA levels in humans are associated with shortening of telomere length. We also hypothesized that shortening of telomere length in peripheral blood is associated with increased breast cancer risk in

women. Accordingly, exposure to high BPA levels may be a risk of breast cancer development in women.

The aims in this study are the following:

AIM I:

a) Explore the association between BPA levels detected in human urine samples with relative telomere length in their peripheral blood.
b) Explore any further association between age, body mass index, menopausal status, current smoking and alcohol drinking statuses with relative telomere length in peripheral blood.

AIM II:

a) Explore the association between relative telomere length in peripheral blood and breast cancer risk in women.
b) Explore any further association between relative telomere length in peripheral blood of breast cancer female patients with characteristics and histological types of breast cancer.

CHAPTER III

SUBJECTS AND METHODS

A. Study population

A Cohort of 501 Lebanese volunteers without cancer of whom 319 were females signed an informed consent. They were recruited from Greater Beirut between February and June 2014. Both females and males were above 18 years old and their fasting urine was collected in glass containers, in order to avoid any exogenous BPA contamination of the urine. Their blood was also withdrawn and stored along with the urine samples at -80ºC. Different data were collected from these volunteers at time of enrollment including: age, weight, height, urinary creatinine, smoking status, alcohol status and menopausal status for females. The female volunteers are used as controls for the subsequent analyses with female breast cancer patients grouped as cases.

A Cohort of 71 female patients with non-metastatic breast cancer were recruited at AUBMC between 2012 and 2013. They provided a written informed consent and their blood was withdrawn before treatment initiation and stored at -80ºC. Information was collected from these patients at time of recruitment includes: age, weight,height,smoking status, alcohol status, menopausal status, ER status, PR status, pathology type, grade, size and lymph node involvement of the breast cancer. Their controls were the non-breast cancer females from the BPA cohort.

B. DNA isolation

DNA was isolated from the peripheral blood of volunteers and breast cancer patients using Qiagen kit and stored at -20 ºC.

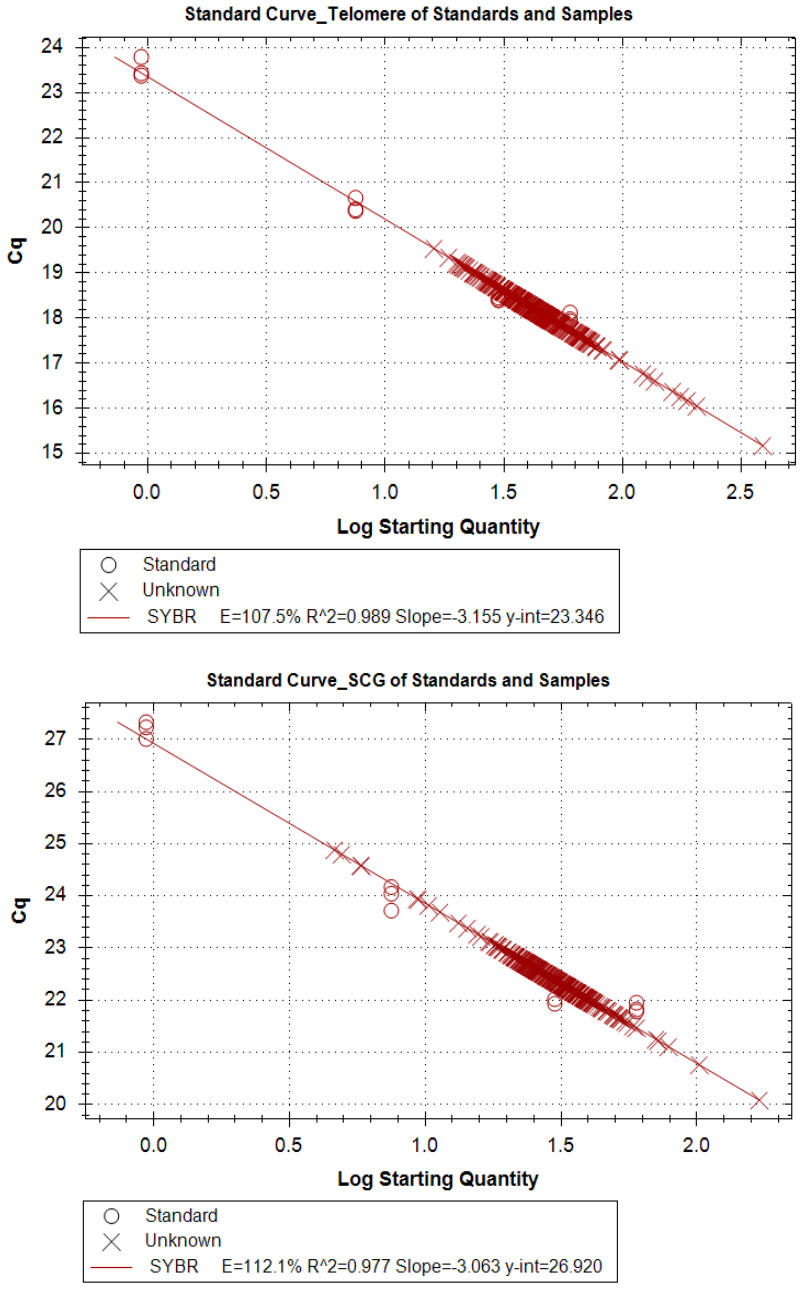
C. RTL quantitative measurement

Telomere length was measured using quantitative real-time polymerase chain reaction (RT-qPCR) using the CFX384 Touch Real-Time PCR Detection System from BIO-RAD as described by Cawthon's method 2002 (Cawthon, 2002). First, DNA samples were diluted to get a concentration of 10 ng/uL, and a sufficient total volume was prepared in order to run both telomere and single copy gene (SCG) PCRs for every sample. The two PCRs were run in a 384-well plates separately, and in each plate, samples were run in triplicates. Telomere mix was prepared using IQ™ SYBR® Green supermix from BIO-RAD along with telomere primers (purchased from TIB MOLBIOL) and DEPC water. For each 10 uL reaction, 30 ng of DNA were added along with 5uL of SYBR green, 1.6 uL of primers: telomere tel c (5'- TgTTAggTATCCCTATCCCTATC CCTATCCCTATCCCTAACA),tel g (5'- ACACTAAggTTTgggTTTggg TTTgggTTTgggTTAgTgT) and 0.4 uL of water. Similarly for SCG mix, for each 10 uL reaction, 30 ng of DNA were added to 5 uL of SYBR green and 2 uL of primers only: hbg1 (5'- gCTT CTgACACAACTgTgTTCACTAgC), hbg2 (5'- CACCAACTTCATCCACgTTCACC). Thermal cycling conditions for telomere were: 50ºC for 2 min, 95ºC for 2 min, then two cycles of 95ºC for 15 sec, 49ºC for 15 sec, then 35 cycles of 95ºC for 15 sec, 62ºC for 10 sec, and 74ºC for 15 sec. For SCG: 50ºC for 2 min,

95ºC for 2 min, then 36 cycles of 95ºC for 15 sec and 58ºC for 1 min. A melt curve was also done to check for reaction specificity and for presence of any primer-dimer formation.

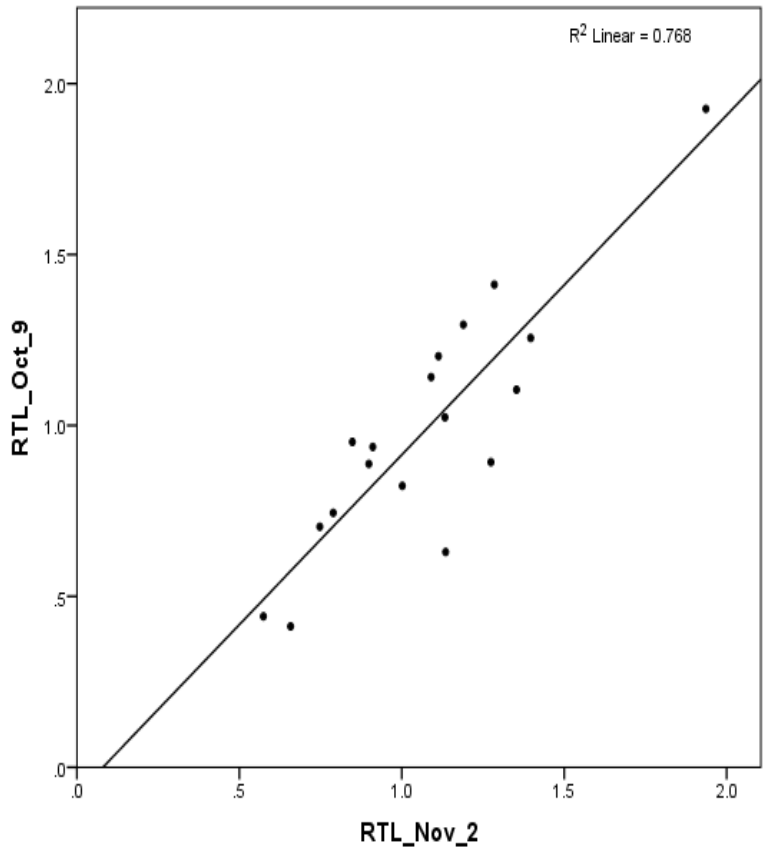
Human β2-globulin gene was used as the single copy gene, it is an internal protein coding gene that normalizes for the starting DNA amount in each sample. Along with the samples, four different standard concentrations of 20 ng/uL, 10 ng/uL, 2.5 ng/uL and 0.3125 ng/uL were prepared from a pool of DNA samples and amplified with samples in each run along with two fixed samples and a negative control to check for contamination. Standard curves were drawn and the slope along with R^2 were calculated using the CFX Manager 3.1 software as shown in **Figure 6**. Intra-assay reproducibility was examined by computing the coefficient of variability of Ct values of telomere and single copy gene for each sample. The intra-assay geometric mean of the coefficients of variation for telomere and single copy gene Ct values were both less than 1.23%. In addition to that, we checked for the inter-run reproducibility by running the telomere and single copy gene PCRs of 18 samples twice. There was a high and statistically significant correlation between the RTL of these samples in the two runs (R^2= 0.768; *P*= 0.00) (**Figure 7**). The equation of the linear regression line is: y=0.994-0.079, showing that the slope is near unity and y-intercept is almost zero, in addition, the inter-assay geometric mean of the coefficient of variation was 6.487 %. Telomere to single copy gene (T/S) ratio was calculated using the formula described by Pfaffl 2001 to account for different plate efficiencies (Pfaffl, 2001). Relative telomere length was calculated by dividing T/S ratio of each sample over that of the standard with the same DNA concentration and it has no unit.

Figure 6. Standard curves for telomere and Human β2-globulin



Standard curves used in order to calculate the relative telomere length. A. represents the standard curve of telomere gene and B. represents the standard curve of Human β2-globulin gene. E is the efficiency of the PCR plate and it is ideally 100%, the R^2 and slope also reflect the plate efficiency which is the fraction of target molecule copied in one PCR cycle.

Figure 7. Correlation between RTL measurements with 18 samples in two RT-PCR independent runs the q-RT PCR



The line shows that RTL values of samples done in October 9 and November 2, 2015 are highly correlated and close to each other with an R^2 equal to 0.768 and a *P*=0.00. This shows that Q-PCR is giving reproducible results.

D. BPA measurement using HPLC-MS

Urinary BPA levels of 497 non cancer volunteers were measured using high performance liquid chromatography-mass spectrometry (HPLC-MS) under Dr. Mouneimne's supervision. The limit of detection was 0.01µg/L. Mean urinary BPA in the whole cohort was 3.67±4.77µg/L with a minimum of 0.01µg/L and a maximum of 59.72 µg/L.

E. Statistical analyses

All data entry and analyses were done using IBM SPSS Statistics v. 23.0 and a $P<0.1$ was considered as statistically significant. Baseline demographics were computed for the whole cohort and shown as mean ± SD or frequencies as appropriate. Data was also computed for the female sub cohort as we are interested to compare their RTL to that of breast cancer female patients.

Creatinine-adjusted urinary BPA levels were calculated by dividing urinary BPA levels over urinary creatinine concentration for every individual so we get the amount of BPA in mg in every 1g of urinary creatinine. This method is used to eliminate any overestimation of BPA levels in participants having a highly concentrated urine sample and avoid underestimation of BPA levels in participants with a diluted urine sample. Although there was a strong correlation between non adjusted and creatinine-adjusted urinary BPA levels ($r= 0.627$, $P< 0.001$), and no statistically significant association between urinary creatinine and RTL tertiles, we chose to analyze the data in 2 ways: one with BPA levels and one with adjusted BPA.

Urinary BPA outliers were calculated using the equation: $IQR*1.5+Q_3$ were IQR stands for interquartile range and Q_3 stands for the 75^{th} percentile (**Figure 8**). After removing outliers, we were left with 458 individuals with a mean urinary BPA level of 2.75±1.6 µg/L. Urinary BPA levels were then categorized into tertiles: T1: <2.33, T2: 2.33-3.49, and T3: >3.49. RTL mean was 1.42 ± 0.85 and also categorized into tertiles: T1: <1.06, T2: 1.06-1.43, T3: >1.43. Similarly in the females sub cohort, the mean of urinary BPA was equal to 2.72±1.69µg/L and urinary BPA and RTL tertiles were found similar to

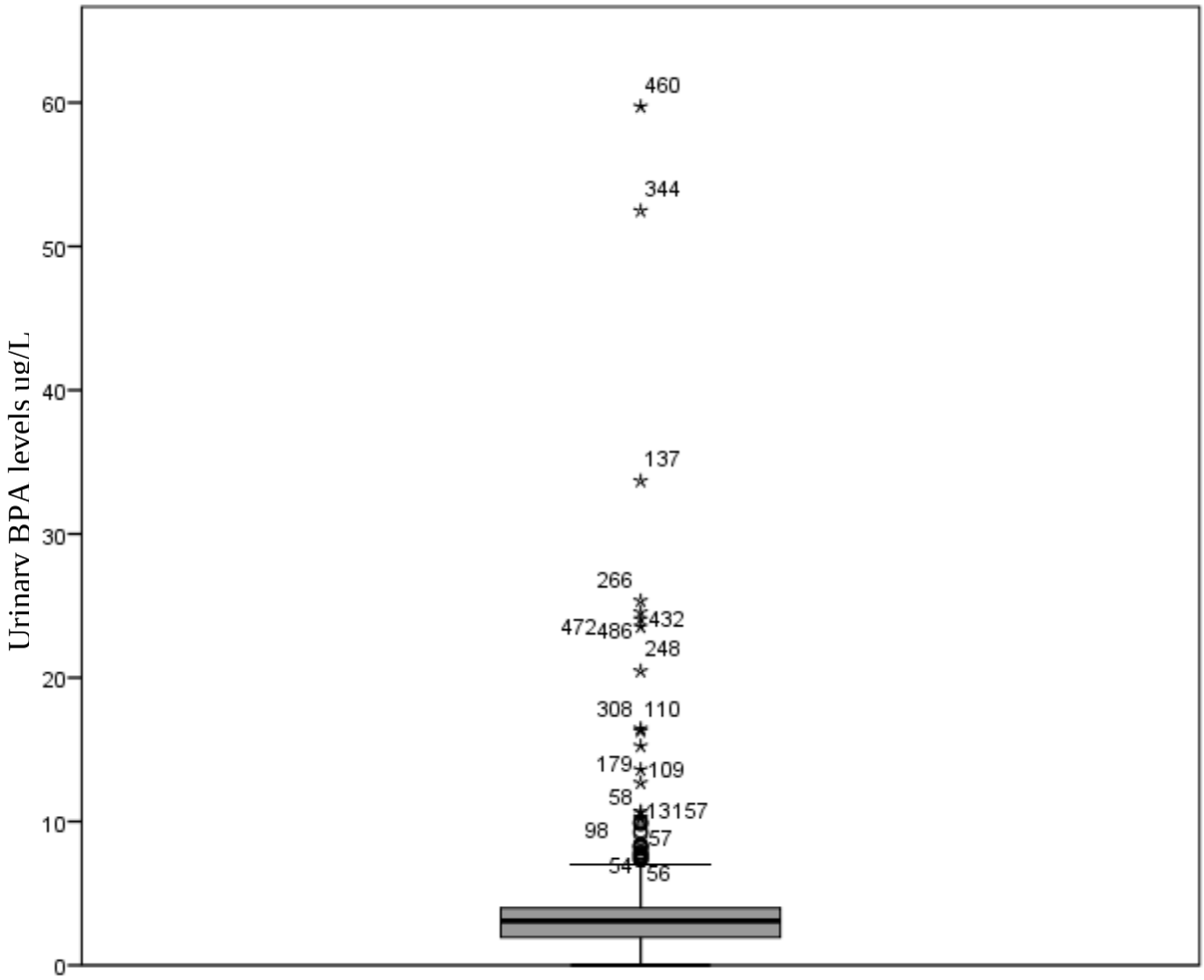
those of the whole cohort after removing outliers. As for the adjusted BPA analysis, outliers were removed using same previous equation and they are shown in the boxplot (**Figure 9)**. We were left with 447 individuals and a mean adjusted BPA urinary concentration equal to 1.81±1.34 µg/g and it was categorized into tertiles: T1: 1.15, T2: 1.15-2.17, T3: >2.17. RTL, with a mean of 1.45 ± 0.87, was also categorized into tertiles: T1: <1.06, T2: 1.06-1.45, T3: >1.45 in the whole cohort after removing outliers. Similarly in the females sub cohort, the mean of urinary BPA was equal to 1.96± 1.43µg/g and urinary BPA and RTL tertiles were found similar to those of the whole cohort after removing outliers.

A univariate multinomial logistic regression model was used to evaluate the association between BPA and RTL tertiles. We calculated the odds ratio (95%CI) of RTL as a linear function of non-adjusted and adjusted BPA urinary levels taking the lowest BPA tertile and the highest RTL tertile as references. The associations between potential confounders and RTL tertiles were analyzed using one-way ANOVA and chi square test as applicable for the whole cohort, after removing outliers, and female sub cohort in non-adjusted and adjusted BPA models. After that, we did initial adjustment for urinary creatinine in case of non-adjusted BPA urinary concentrations, and followed with full adjustment for variables that appeared to be confounders: age, BMI and menopausal status in women.

In the breast cancer cohort, peripheral blood RTL was used as a continuous variable. The differences in the baseline demographics between controls and cases were analyzed using independent-sample t test and chi square test as applicable. Binomial

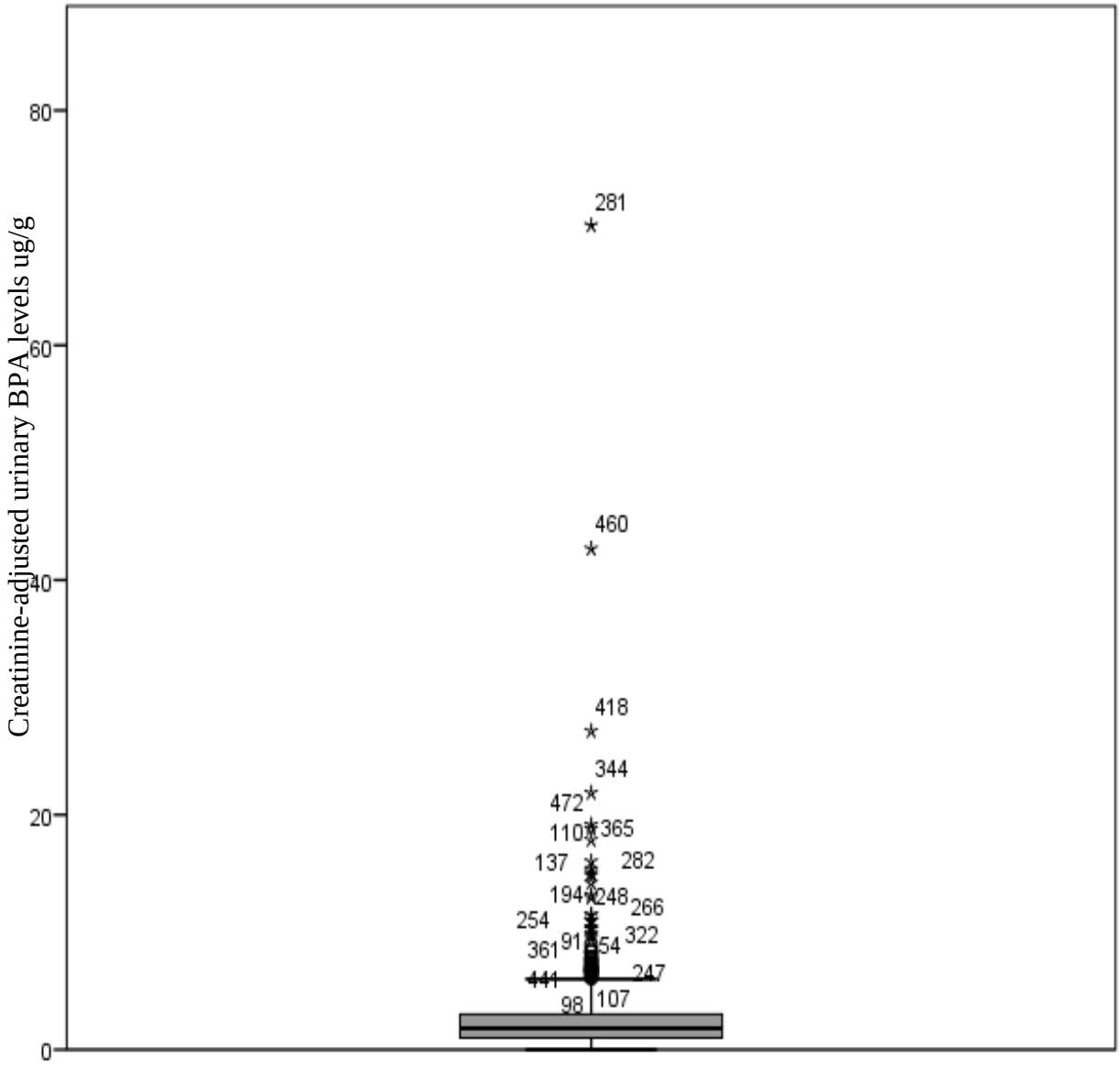
logistic regression was used to analyze the association between RTL and breast cancer risk, and it was adjusted for confounding variables, basically: age, current smoking and drinking alcohol status. The cancer characteristics were also described and their potential association with RTL was evaluated using ANOVA or t-test as applicable.

Figure 8. Box plot and outliers of urinary BPA concentrations (µg/L) in the whole cohort



Boxplot showing the median of urinary BPA levels in the whole cohort and the outliers that reside above the whiskers. 39 outliers out of 497 samples were calculated using the interquartile range.

Figure 9. Box plot and outliers of creatinine-adjusted urinary BPA levels (µg/g) in the whole cohort



Boxplot shows the median of creatinine-adjusted urinary BPA levels in the whole cohort and the outliers that reside above the whiskers. The outliers were calculated using the interquartile range and were found to be 50 outliers

CHAPTER IV

RESULTS

Out of the 501 Lebanese individuals recruited, we were able to measure RTL for 497 individuals. By calculating urinary BPA or creatinine-adjusted urinary BPA outliers, we ended up having 458 and 447 individuals respectively. Subsequent analyses were done on both models in order to study the association between BPA levels and RTL. In addition to that, the association between RTL and breast cancer risk was also studied on blood samples of breast cancer patients.

A. Characteristics of the controls and cases

Table 7 represents the different baseline demographics of the whole cohort, after removing outliers, and also those of males and females subcohorts. The mean age of subjects was 45.5± 15.11 years old whereby men were younger than females by 5 years (42.05 ±16.64 versus 47.49 ±13.8). The whole cohort, after removing outliers, was overweight with a mean BMI of 29.12 ±5.79 Kg/m^2 and a mean BMI of 28.01 ±1.43 $Kg/m^2$and 29.76 ±6.02 Kg/m^2inmales and females sub cohorts respectively. Mean BPA urinary levels in the whole cohort, after removing outliers, was 2.75 ±1.6 µg/L which is similar to that of females 2.72 ±1.69 µg/L and slightly higher in males 2.81 ±1.43 µg/L. Less than half of the whole cohort, after removing outliers, was current smoker whereby males were 54.5% current smokers compared to 37.5% of females. In addition to that, most

subjects were not current alcohol drinkers whereby only 19.9% currently drink alcohol and most of them were males (43.1%) and only 6.5% of females. **Table 8** represents the baseline demographics of the whole cohort, after removing outliers, and also of males and females subcohorts after creatinine adjustment of the urinary BPA levels.

Out of the 71 breast cancer female patients, 68 of them had their RTL measured, and their characteristics compared to those of 319 females from the BPA cohort as shown in **Table 9**. There was a significant difference in the mean age between cases and controls (52.83±12.86 years versus 47.34±13.91 years). In addition to that, both percentages of current smokers and alcohol drinkers were 22.1% in cases versus 37.3% and 6.6% respectively in controls.

Table 7. Baseline demographics of the whole cohort and of the males and females subcohort respectively after the removal of 39 urinary BPA outliers

	Total	**Males**	**Females**
Volunteers	458	167	291
Age in years	45.50±15.11	42.05±16.64	47.49±13.80
BMI ($Kg/m^{2)}$	29.12±5.79	28.01±5.20	29.76±6.02
BPA µg/L	2.75±1.60	2.81±1.43	2.72±1.69
Urinary creatinine mg/dL	173.86±89.91	216.56±93.24	149.36±78.17
Current Smokers**Yes**	200 (43.7)	91 (54.5)	109 (37.5)
Current Alcohol drinkers **Yes**	91 (19.9)	72 (43.1)	19 (6.5)
Postmenopausal status **Yes**	-	-	136 (46.7)

Mean±standard deviation, number of subjects (percentages)

Table 8. Baseline demographics of the whole cohort and of the males and females subcohort respectively after the removal of 50 creatinine-adjusted urinary BPA outliers

	Total	**Males**	**Females**
Volunteers	447	169	278
Age in years	45.02±15.06	41.78±16.58	46.99±13.72
BMI Kg/m^2	29.09±5.87	27.94±5.24	29.79±6.12
BPA µg/L	2.87±1.88	3.003±1.82	2.78±1.91
Urinary creatinine mg/dL	182.46±85.97	220.95±89.65	159.07±74.65
Serum creatinine mg/dL	0.77±0.23	0.93±0.2	0.67±
Adjusted urinary BPA µg/g	1.81±1.34	1.55±1.13	1.97±1.43
Current Smokers **Yes**	197 (44.1)	91 (53.8)	106 (38.1)
Current Alcohol drinkers **Yes**	89 (19.9)	71 (42)	18 (6.5)
Postmenopausal status **Yes**	-	-	125 (45)

Mean±standard deviation, number of subjects (percentages)

Table 9. Comparison of baseline characteristics between breast cancer females (cases) and non-breast cancer females from the BPA cohort (controls)

	Cases	**Controls**	***P***
Characteristics	68	319	
Age in years	52.83±12.86	47.34±13.91	**0.003**[1]
BMI Kg/m^2	28.23±6.69	29.69±6.03	0.117[1]
Current Smokers Yes	15 (22.1%)	119 (37.3%)	**0.034**[2]
Current Alcohol drinkers Yes	15 (22.1%)	21 (6.6%)	**0.00**[2]
Postmenopausal status Yes	34 (50%)	148 (46.4%)	0.384[2]

[1]Independent sample t-test, [2] Chi square test, mean±standard deviation, number of subjects (percentages)

Cancer properties for female patients with breast cancer are shown in **Table 10.**The majority of patients had invasive ductal carcinoma (80.9%), ERα (77.9%) and PR (70.6%) positive status. Most had grade 2 breast cancer (36.8%), tumor size of 2 cm or less (T1: 57.4%), no lymph nodes involvement (60.3%), and none of the patients had metastasis.

Table10. Breast cancer characteristics of cases

		Total
Characteristics		68
Pathology type	IDC	55(80.9%)
	ILC	5 (7.4 %)
	Both	3 (4.4%)
	Other	1 (1.5%)
ER status: Positive		53(77.9%)
PR status: Positive		48(70.6%)
Grade	1	17 (25%)
	2	25(36.8%)
	3	17 (25%)
T	0	1 (1.5%)
	1	39(57.4%)
	2	23(33.8%)
	3	1 (1.5%)
N	0	41(60.3%)
	1	20(29.4%)
	3	1(1.5%)

[1]: one-way ANOVA, [2]: chi square test, $P<0.05$ when compared to <1.06 (post hoc test bonferroni), number of subjects (percentages)

B. Association between urinary BPA levels and relative telomere length

In the whole BPA cohort, we observed a statistically significant association between age and RTL tertiles (*P*= 0.001) and the association between BMI and RTL tertiles was statistically significant (*P*= 0.08). There were no statistically significant associations between gender, current smoking status, and current alcohol drinking status with RTL tertiles as shown in **Table 11**. Similar statistically significant associations were found within the females subjects. Age (*P*= 0.017), BMI (*P*= 0.057) and menopausal status (*P*= 0.008) were associated with RTL tertiles within the females sub cohort. Chi square (x^2) analysis between BPA tertiles and RTL tertiles in the whole BPA cohort showed a trend towards having higher BPA urinary levels associated with shorter RTL and lower BPA urinary levels associated with longer RTL, however these findings were not statistically significant *P*= 0.328 (**Figure 10**). However, by looking at the female sub cohort alone, this trend became statistically significant *P*= 0.022 (**Figure 11)**. Univariate multinomial Logistic regression model showed that high urinary BPA level was less likely to be associated with long RTL and more likely to be associated with the short RTL (OR= 2.08, 95%CI= 1.02-4.23, *P*= 0.043) in females sub cohort only but not in the whole BPA cohort. After initial adjustment for urinary creatinine levels, similar findings were observed with high urinary BPA levels with a *P* of 0.283 and 0.028 in the whole cohort, after removing outliers, and female subcohort respectively. After full adjustment for urinary creatinine, age, BMI, and postmenopausal status, the association between high urinary BPA levels and short RTL tertile was still statistically significant having OR= 1.98, 95%CI= 0.95-4.13, *P*= 0.067 as shown in **Table 12.**

Table 11. Association between baseline demographics of the whole cohort without urinary BPA outliers (N= 458)

		RTL			*P*-value
		<1.06	1.06-1.43	>1.43	
Urinary creatinine mg/dL		168.9±86.95	171.9±91.11	180.8±91.74	0.485[1]
Age in years		48.99±14.42	44.59±15.06	42.95±15.29	**0.001**[1]
BMI Kg/m^2		29.92±5.75	28.96±5.61	28.48±5.96	**0.08**[1]
Gender	Males	57(37.5)	59 (38.3)	51 (33.6)	0.653[2]
	Females	95 (62.5)	95 (61.7)	101 (66.4)	
Current Smokers **Yes**		69 (45.4)	74 (48.1)	57 (37.5)	0.154[2]
Current Alcohol drinkers **Yes**		32 (21.1)	34 (22.1)	25 (16.4)	0.422[2]
Postmenopausal status **Yes**		55 (57.9)	45 (47.4)	36 (35.6)	**0.008**[2]

[1]: one-way ANOVA, [2]: chi square test, p-value <0.05 when compared to <1.06 (post hoc test bonferroni), mean±SD, number of subjects (percentage)

Figure 10. Distribution of RTL tertiles among BPA tertiles in all subjects *P*= 0.328

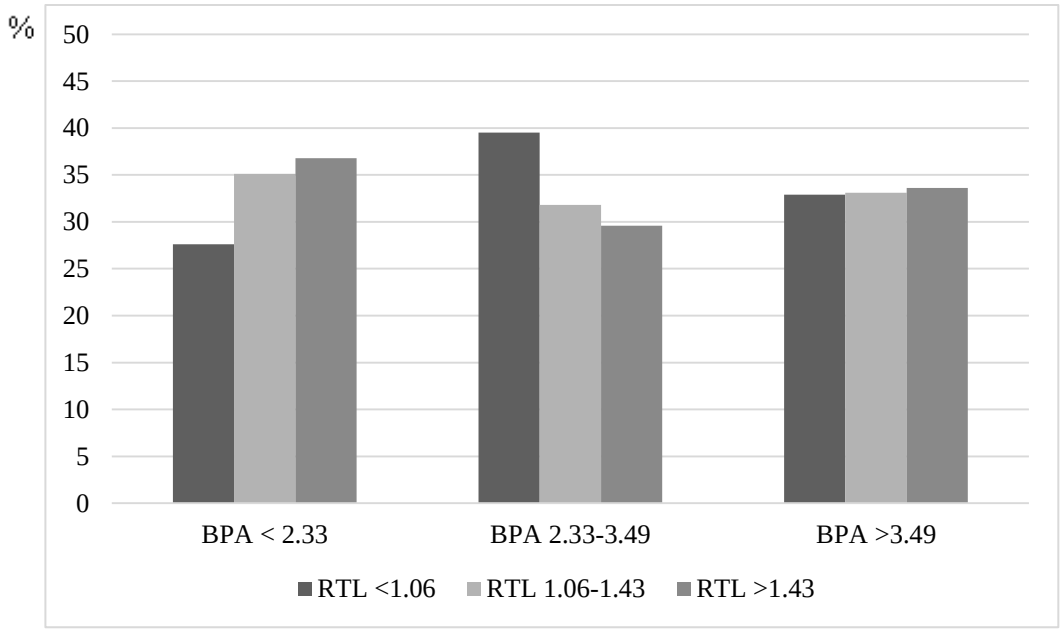


Figure 11. Distribution of RTL among BPA tertiles in females only, *P*= 0.022

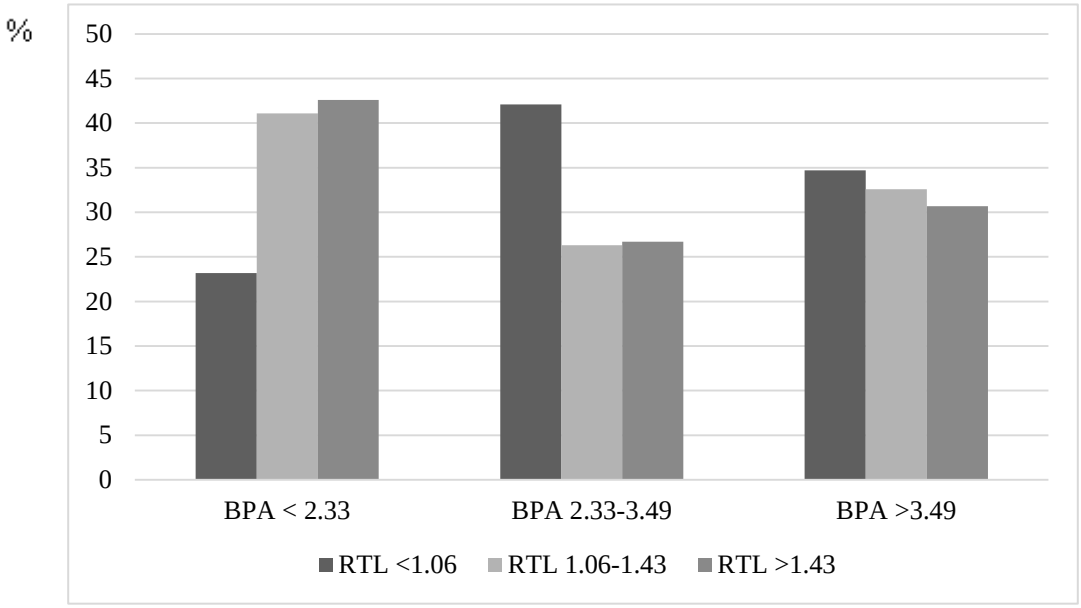


Table 12. Association of BPA with RTL tertiles using a univariate and multivariate logistic regression model

		Univariate				Multivariate*				Multivariate#			
		RTL< 1.06		RTL 1.06 - 1.43		RTL< 1.06		RTL 1.06 - 1.43		RTL< 1.06		RTL 1.06 - 1.43	
	BPA levels	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
All	>3.49	1.31(0.75-2.29)	0.347	1.04(0.61-1.78)	0.895	1.36(0.78-2.39)	0.283	1.07(0.62-1.84)	0.817	1.27(0.72-2.25)	0.414	1.05(0.61-1.82)	0.852
	2.33- 3.49	1.78(1.019-3.1)	**0.043**	1.13(0.65-1.96)	0.665	1.82(1.04-3.18)	**0.036**	1.15(0.66-1.99)	0.629	1.87(1.06-3.3)	**0.03**	1.16(0.67-2.01)	0.603
	<2.33	1	-	1	-	1	-	1	-	1	-	1	-
Female	>3.49	2.08(1.02-4.23)	**0.043**	1.1(0.57-2.13)	0.772	2.24(1.09-4.59)	**0.028**	1.16(0.6-2.26)	0.663	1.98(0.95-4.13)	**0.067**	1.1(0.56-2.17)	0.775
	2.33- 3.49	2.9(1.42-5.88)	**0.003**	1.02(0.51-2.05)	0.954	3.03(1.48-6.19)	**0.002**	1.05(0.52-2.12)	0.887	2.83(1.37-5.85)	**0.005**	1.02(0.5-2.06)	0.959
	<2.33	1	-	1	-	1	-	1	-	1	-	1	-

*adjusted for urinary creatinine, #adjusted for urinary creatinine, age and BMI (in addition to postmenopausal status in females)

C. Association between creatinine-adjusted urinary BPA levels and relative telomere length

In the whole BPA cohort and after removing adjusted BPA outliers, we also observed a statistically significant association between age and current smoking status with RTL tertiles (*P*= 0.001, and *P*= 0.019 respectively). The association was statistically significant between BMI and RTL tertiles with a *P*= 0.088. There were no statistically significant associations between gender and current alcohol drinking status with RTL tertiles as shown in **Table 13**. Within the females sub cohort, age (*P*= 0.01), BMI (*P*= 0.071) and menopausal status (*P*= 0.004) were statistically significantly associated with RTL tertiles. Chi square (x^2) analysis between adjusted BPA tertiles and RTL tertiles in the whole BPA cohort showed a trend towards having higher BPA urinary levels associated with shorter RTL and lower BPA urinary levels associated with longer RTL, however these findings were not statistically significant *P*= 0.453 (**Figure 12**). By looking at the female subcohort alone, this trend was shown to be statistically significant with a *P*= 0.038 (**Figure 13)**. Univariate multinomial Logistic regression model showed that high adjusted BPA urinary levels were more likely to be associated with short RTL and less likely to be associated with the long RTL (OR=2.794,95%CI=1.36-5.74, *P*= 0.005) in females subcohort only, but not in the whole BPA cohort. After full adjustment for age, BMI, and menopausal status, the association between BPA tertiles and RTL tertiles was still not statistically significant but it remained statistically significant in the case of the female sub cohort with OR=2.228, 95%CI= 1.06 – 4.7, *P*= 0.036 as shown in **Table 14.**

Table 13. Association of baseline characteristic of the whole cohort without creatinine-adjusted urinary BPA outliers with RTL

	RTL			*P*
	<1.06	1.06-1.45	>1.43	
Age in years **(mean ±SD)**	48.82±14.72	43.7±14.58	42.54±15.22	**0.001**[1]
BMI Kg/m^2**(mean ±SD)**	29.93±5.81	28.88±5.7	28.47±6.04	**0.088**[1]
Gender **N (%)**Males	58(38.9)	59 (39.6)	52 (34.9)	0.664[2]
Females	91 (61.1)	90 (60.4)	97 (65.1)	
Urinary creatinine mg/dL **(mean±SD)**	177.13±85.9	178±84.27	190.27±87.76	0.383[1]
Current Smokers**Yes N (%)**	67 (45)	77 (51.7)	53 (35.6)	**0.019**[2]
Current Alcohol drinkers**Yes N (%)**	31 (20.8)	35 (23.5)	23 (15.4)	0.208[2]
Postmenopausal status **Yes N (%)**	52 (57.1)	41 (45.6)	32 (33)	**0.004**[2]

[1]: one-way ANOVA, [2]: chi square test, $P<0.05$ when compared to <1.06 (post hoc test Bonferroni)

Figure 12. Percentage distribution of RTL by BPA adjusted to creatinine tertiles among all, $P= 0.453$

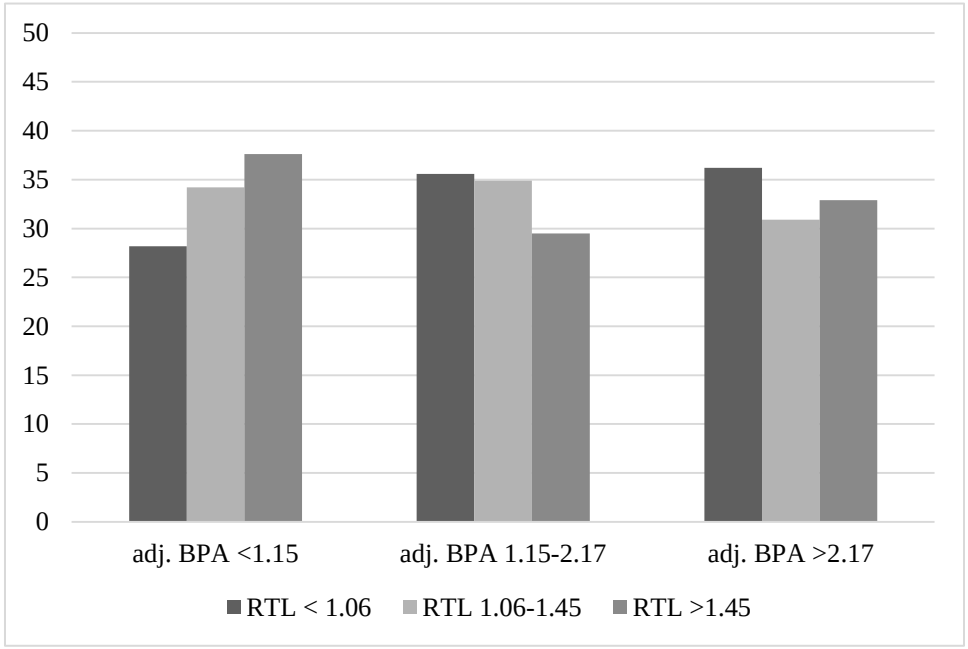


Figure 13. Distribution of RTL among adjusted BPA tertiles in females only ***P***=0.034

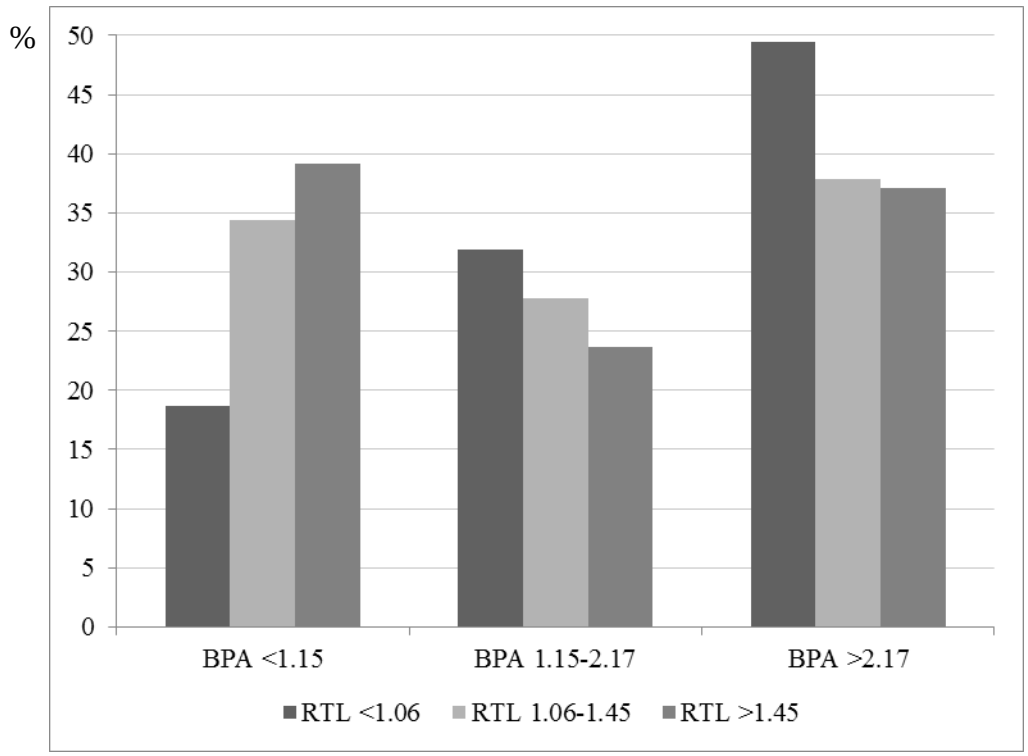


Table 14. Association of adjusted urinary BPA tertiles with RTL tertiles using a univariate and multivariate multinomial logistic regression model

		Univariate				**Multivariate**			
		RTL< 1.06		RTL 1.06 - 1.45		RTL< 1.06		RTL 1.06 - 1.45	
		OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
All*	>2.17	1.469 (0.84-2.56)	0.175	1.031 (0.59-1.79)	0.914	1.206 (0.68-2.14)	0.522	0.985 (0.56-1.73)	0.958
	2.17- 1.15	1.606 (0.91-2.83)	0.101	1.298 (0.75-2.26)	0.355	1.665 (0.94-2.96)	0.083	1.307 (0.75-2.27)	0.342
	<1.15	1		1		1		1	
	>2.17	**2.794 (1.36-5.74)**	**0.005**	1.158 (0.59-2.26)	0.667	2.228 (1.06-4.7)	**0.036**	1.037 (0.52-2.06)	0.918
Females [#]	2.17- 1.15	2.818 (1.28-6.22)	0.1	1.332 (0.64-2.79)	0.446	2.794 (1.24-6.27)	**0.013**	1.319 (0.627-2.77)	0.465
	<1.15	1		1		1		1	

*Multivariate: BMI, age, [#] multivariate: BMI, age, menopausal status

D. Association between RTL and breast cancer risk

The mean RTL of females controls was 1.42 ± 0.76 compared to cases with a mean RTL of 0.41 ± 0.1 and the difference was statistically significant using independent sample t-test with a *P* of 0.00, whereby breast cancer females have shorter mean RTL than that of controls (**Figure 14**). Binary logistic regression showed that with each one unit increase in RTL, it is less likely to have breast cancer with OR= 0.25, 95%CI= 0.167-0.375, *P*= 0.00. By comparing the different baseline characteristics of controls and cases, we found that age (*P*= 0.003), current smoking status (*P*= 0.034) and current alcohol drinking status (*P*= 0.00) were statistically significantly different and we needed to adjust for them. After adjustment for age, current smoking status, and current alcohol drinking status, the difference in RTL was still statistically significant whereby for each one unit increase in RTL, it is less likely to have breast cancer with OR= 0.214, 95%CI= 0.128-0.358, *P*= 0.00(**Table 15**).

Figure 14. Comparison of mean RTL of non-breast cancer (N=319) versus breast cancer female patients (N=68)

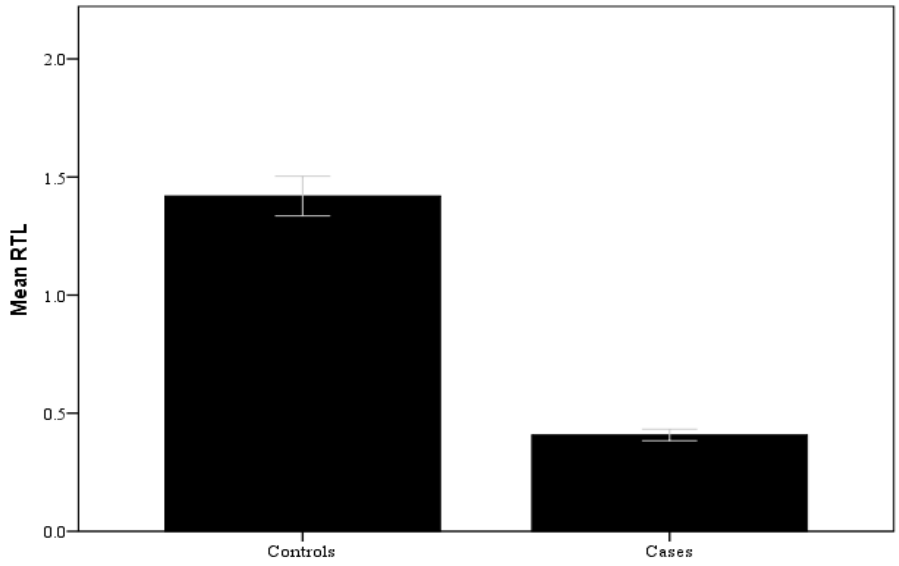


Table 15. Unadjusted and adjusted odds ratio of breast cancer risk results of the univariate and multivariate binary logistic regression model

	Breast cancer			
	Univariate		Multivariate[#]	
RTL	OR (95% CI)	*P*	OR (95% CI)	*P*
	0.25 (0.167-0.375)	**0.00**	0.214 (0.128-0.358)	**0.00**

[#] Multivariate binary logistic regression model adjusted for age, current smoking status and current alcohol drinking status

E. Association between age and RTL

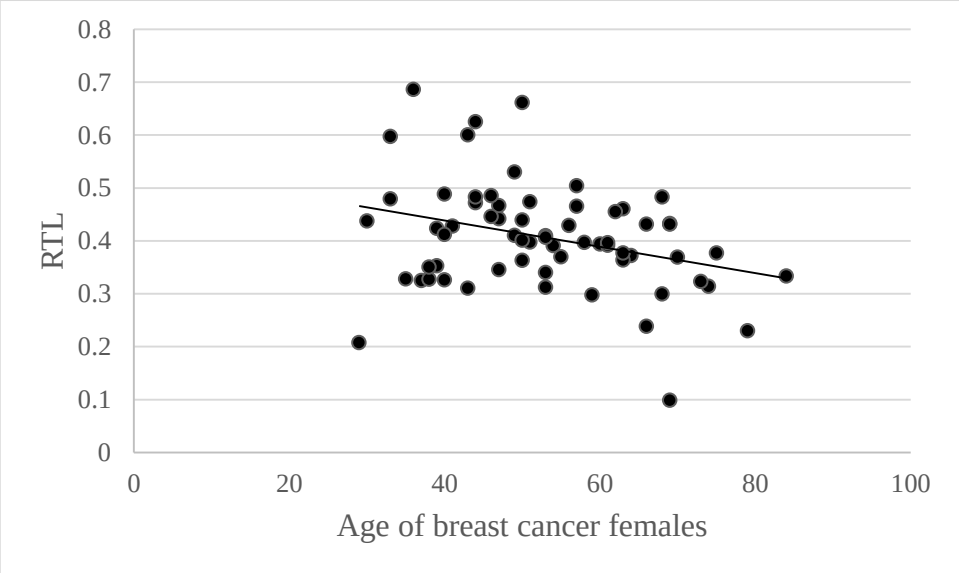
All baseline demographics and cancer characteristics in the female breast cancer patients were analyzed to investigate any association with RTL. Only age was found to be statistically significantly associated with RTL (*P*= 0.01) and menopausal status was statistically significant with a *P*= 0.076, yet all other demographics and cancer characteristics were not significantly associated with RTL. (**Table 16, Figure 15**).

Older breast cancer patients had shorter relative telomere length in their peripheral blood compared to younger ones. In the whole BPA cohort, it was also shown that older women and men have shorter peripheral blood RTL than younger ones with a *P*= 0.025 and *P*= 0.034 in males and females subcohorts respectively. This shortening in telomere length was shown to be accelerated in the breast cancer women whereby the curve showed a steeper line.

Table 16. Association between breast cancer characteristics and RTL

	RTL	
	B	***P***
Age	-0.317	**0.01**
BMI	-0.155	0.234
Smoking Status	0.049	0.702
Alcohol status	0.137	0.282
Postmenopausal	-0.222	**0.076**
ER status	0.117	0.355
PR status	0.151	0.233
Grade	-0.014	0.757
Pathology type	-0.135	0.288
T	-0.095	0.453
N	0.091	0.48

Figure15. Correlation between ageand RTL in breast cancer patients (*P*= 0.01)



This line represents the correlation between age and relative telomere length in the breast cancer cohort with a line eqaution:y= -0.0025x+0.5381, R^2= 0.1002.

CHAPTER IV

DISCUSSION AND CONCLUSION

In this study, we found that high levels of non-adjusted and creatinine adjusted urinary BPA were significantly associated with shorter relative telomere length in the female subcohort. To our knowledge, this is the first study to evaluate the association of BPA levels with relative telomere length of peripheral blood in humans, and there is an in vitro study on MCF-7 cells, showing that BPA induces telomeric associations that are due to telomere loss causing chromosome end fusions (Roy et al., 1998; Bayne et al., 2008).Moreover, a study on MDA-MB-231 ERα negative cell lines, showed that BPA induced DNA damage through reactive oxygen species (ROS) production, and up-regulated c-Myc, which is a cell-cycle regulator protein, leading to increased cell proliferation. Both increased ROS production and cellular proliferation may contribute to telomere shortening (Pfeifer, Chung, & Hu, 2015). Another interesting study done on *C. elegans*, a nematode with homologues identified for 60-80 % of human genes (Kaletta & Hengartner, 2006), showed that treating with low doses of BPA caused a down-regulation in *mrt-2* checkpoint gene that is involved in telomere replication, and hence its down-regulation causes loss in telomere repeats and increase in chromosomes end fusion (Allard & Colai+ícovo, 2010). In addition, aging is associated with shorter relative telomere length which may be a confounding factor for menopausal status.

We also found that short relative telomere length in peripheral blood of breast cancer females is associated with increased breast cancer risk. These results were consistent with other studies. For example, Shen et al. found that the shortest peripheral

blood telomere quartile of younger or pre-menopausal women was associated with increased risk of breast cancer risk in a large cohort of 1,108 controls and 1,061 cases, and this study used the same method we adopted for measuring relative telomere length (Shen et al., 2009). Similar results were seen in Levy et al.'s study which was the first to show the association between short telomere length of white blood cells and breast cancer risk using Southern blot assay, but no association was seen between age and relative telomere length (Levy et al., 1998).

Telomeres are very important for the genomic stability, thus reduction in telomere length causes an increase in genomic mutations and chromosomes end fusion. Telomere reduction takes place during each cell division in human somatic cells that have repressed telomerase activity. When the telomere length reaches a critical level, the cell must die in order to avoid any further threat to the genome, and this phase is known as telomere crisis. A study found that the telomere crisis takes place during the transition from usual ductal hyperplasia (UDH) to ductal carcinoma in-situ (DCIS) which is accompanied with an increase in genomic instabilities and incidences of anaphase bridges. That is why shorter telomere length was found in (DCIS) tissues compared to (UDH), and even shorter telomere length was found in invasive cancer tissues. Although telomerase is activated in cancer cells, short telomere length may be due to the fact that telomerase is acting only on the chromosomes with the shortest telomere length rather that stabilizing the whole telomeric subsets (Chin et al., 2004).

There are also other mechanisms that may explain these findings. For instance, it was found that shortening of telomere length was associated with an increase in the expression of telomere-associated proteins such as TRF1, TRF2, or TIN2 in breast cancer tissues. These proteins bind to the telomeric end, hence inhibiting telomerase

access. Interestingly, these telomere-associated proteins were found to be up-regulated during stress and inflammation. This can be a potential pathway for BPA to shorten telomere length, since in in-vivo and human association studies, BPA was found to increase oxidative stress, corticosterone levels and inflammatory markers (Yang et al., 2009; Poimenova et al., 2010). BPA acts on glucocorticoid receptors (Sargis et al., 2010).

Furthermore, two signaling pathways are very important for regulating cell cycle and proliferation. The first pathway is the *p53/p21* pathway, *p53* being a tumor protein and *p21* a cyclin-dependent kinase inhibitor. Both proteins regulate cell cycle leading to cellular apoptosis. When this pathway is halted, the cell continues on replicating regardless of critical telomere shortening. The other pathway is the *p16/RB1* pathway, whereby *p16* being another type of cyclin-dependent kinase inhibitor that avoids the inactivation of the retinoblastoma protein (*pRb*). When this pathway is inactivated, the cells no more progress from the G1 phase into the S phase, and thus leading to cell cycle arrest which may be an early event in breast cancer progression. The end result of cells escaping these two pathways is further shortening in relative telomere length, activation of telomerase enzyme, and subsequent genomic alterations leading to a cancerous cell. It was found that hyper methylation at the promoter sites of *TP53*, *P21*, and *P16* genes was significantly associated with shorter telomere length in breast cancer tissues compared to adjacent normal ones (Radpour et al., 2010). Moreover, shorter telomere length in breast cancer blood and tissues is due to the increased proliferative capacity of breast cancer cells and these cells might be exhibiting lower telomerase activity than cancer cells with longer telomere length (Chin et al., 2004).

Despite the above positive results and their potential explanations, others did not find any association between relative telomere length and breast cancer risk (BARCZAK et al., 2016a; De, I et al., 2009; Zheng et al., 2010). These results raised an important question whether one should be looking at the length of the 92 telomeres in the whole chromosomes, or look at specific ones. This is because it was shown that chromosome end fusion in breast cancer does not happen randomly; as a matter of fact, certain chromosome arms such as: 9p, 15p, 15q and Xp were found to be frequently abnormal in blood of breast cancer pre-menopausal women(Zheng, Zhou, Loffredo, Shields, & Sun, 2011).

To make things even more complicated, and contrary to our results, others found an association between longer relative telomere length in peripheral blood and breast cancer risk (Svenson et al., 2008; Gramatges et al., 2010). For example, Svenson et al. showed that longer telomere length in the blood of breast cancer patients could be due to the high telomerase activity in breast cancer cells after telomere crisis occurs. In addition, the brother of the regulator of imprinted sites (BORIS), was found to be up-regulated in cancer cells. BORIS acts by inhibiting CTFC (CCCTC-binding factor) that negatively regulates hTERT expression and hence BORIS indirectly activates telomerase enzymes. Moreover, circulating cytokines that can activate telomerase such as: interleukin 2 (IL-2),IL-4, IL-6, IL-7, IL-10, and IL13 were found to be higher in breast cancer females serum compared with controls. However, Svenson et al. was criticized for measuring the relative telomere length of the cases and some of the controls from the buffy coat of their blood samples while using granulocytes for measuring telomere length of the remaining controls (1/3). Granulocytes normally have shorter telomere length compared to lymphocytes in buffy coat and thus this may be the

cause of long relative telomere length association with breast cancer (Svenson et al., 2008).

Herein, we present the first study that evaluates the association between urinary BPA levels and relative telomere length in peripheral blood of humans. Moreover, it is the first study in Lebanon to evaluate the association between relative telomere length and breast cancer risk using peripheral blood before treatment initiation. The BPA cohort consisted of a large sample size (around 500 individuals) and hence a better representation of the Lebanese population. Quantitative real time PCR was used for measuring relative telomere length from the peripheral blood. It is an economical, high-throughput, and sensitive method that measures telomere length from nanograms of DNA. In contrast, to telomere restricted fragment assay by Southern blot, the method we used does not measure the sub-telomeric part of the DNA, which usually varies between individuals (Cawthon, 2002).

Despite the strength, the study suffers from some limitations. For instance we did not measure the 24-h urinary output volume, and hence we could not calculate the daily intake of BPA (ng/kgday) in the Lebanese individuals according to this equation: urinary BPA (ng/ml)* urinary output (ml/day)/body weight (kg) (LaKind & Naiman, 2011). Moreover, there are other residual and uncollected confounders that may have an effect on telomere length but not taken into account. Another thing is that the BPA levels were not measured in the breast cancer cohort. A major limitation in the breast cancer cohort is that the peripheral blood was collected after diagnosis with breast cancer and before treatment initiation where telomere shortening may be due to the influence of breast cancer itself and not a risk of getting breast cancer.

Further experimental plans, we are planning to measure relative telomere length from breast cancer and adjacent normal tissues. We are also in the process of measuring relative telomere length in cell-free circulating DNA that is shed from cancerous cells. For instance in this study, relative telomere length was measured from peripheral blood that includes leukocytes and cell-free circulating DNA and it would be interesting to look at cell-free circulating DNA alone. In addition, subsequent in-vitro studies are to be done on different cell lines such as MCF-10A (human breast epithelial cell line), MCF-7 (an estrogen receptor positive human breast cancer cell line) and MDA-MB-231 cells (an estrogen receptor negative human breast cancer cell line) to test the effect of BPA on telomere length with and without an estrogen inhibitor to validate whether the potential effect of BPA on telomere length takes place through binding to the estrogen receptor. Finally, we are planning to measure telomerase activity as well in breast cancer females' peripheral blood and tissues and in cancer cell lines.

In conclusion, this is the first study to show an association between high urinary BPA levels and shortening of telomere length in peripheral blood of Lebanese women. In addition, shorter telomere length in peripheral blood was associated with an increase in breast cancer risk in Lebanese women. Many studies have found that BPA is capable of inducing tumorigenesis in mammary glands in animal models and short telomere length was found to increase the risk of breast cancer. BPA can thus be a potential risk of breast cancer and telomere length may be recognized as a future biomarker for breast cancer diagnosis and prognosis which needs in both cases further studies to be done.

BIBLIOGRAPHY

http://www.bisphenol-a.org/. (2016).

Ref Type: Online Source

Acevedo, N., Davis, B., Schaeberle, C. M., Sonnenschein, C., & Soto, A. M. (2013). Perinatally administered bisphenol a as a potential mammary gland carcinogen in rats. *Environ.Health Perspect., 121,* 1040-1046.

Allard, P. & Colai+ícovo, M. P. (2010). Bisphenol A impairs the double-strand break repair machinery in the germline and causes chromosome abnormalities. *Proceedings of the National Academy of Sciences of the United States of America, 107,* 20405-20410.

American Cancer Society (2012). *Breast Cancer Facts & Figures 2011-2012* Atlanta.

American Cancer Society (2016). *Cancer Facts & Figures* Atlanta.

Andersen, H. R., Andersson, A. M., Arnold, S. F., Autrup, H., Barfoed, M., Beresford, N. A. et al. (1999). Comparison of short-term estrogenicity tests for identification of hormone-disrupting chemicals. *Environmental Health Perspectives, 107,* 89.

Antoniou, A., Pharoah, P. D. P., Narod, S., Risch, H. A., Eyfjord, J. E., Hopper, J. L. et al. (2003). Average Risks of Breast and Ovarian Cancer Associated with BRCA1 or BRCA2 Mutations Detected in Case Series Unselected for Family History: A Combined Analysis of 22 Studies. *American Journal of Human Genetics, 72,* 1117-1130.

Augustine, T. A., Baig, M., Sood, A., Budagov, T., Atzmon, G., Mariadason, J. M. et al. (2015). Telomere length is a novel predictive biomarker of sensitivity to anti-EGFR therapy in metastatic colorectal cancer. *British Journal of Cancer, 112,* 313-318.

Babizhayev, M. A., Savel'yeva, E. L., Moskvina, S. N., & Yegorov, Y. E. (2011). Telomere Length is a Biomarker of Cumulative Oxidative Stress, Biologic Age, and an Independent Predictor of Survival and Therapeutic Treatment Requirement Associated With Smoking Behavior. *American Journal of Therapeutics, 18*.

Bae, B., Jeong, JH., & Lee, SJ. (2002). The quantification and characterization of endocrine disruptor bisphenol-A leaching from epoxy resin. *water science and technology, 46,* 381-387.

BARCZAK, W. O. J. C., ROZWADOWSKA, N. A. T. A., ROMANIUK, A. L. E. K., LIPI+âSKA, N. A. T. A., LISIAK, N. A. T. A., GRODECKA-GAZDECKA, S. Y. L. W. et al. (2016a). Telomere length assessment in leukocytes presents potential diagnostic value in patients with breast cancer. *Oncology Letters, 11,* 2305-2309.

Bayne, S., Jones, M. E., Li, H., Pinto, A. R., Simpson, E. R., & Liu, J. P. (2008). Estrogen deficiency leads to telomerase inhibition, telomere shortening and reduced cell proliferation in the adrenal gland of mice. *Cell Res., 18,* 1141-1150.

BAYNE, S. H. A. R., JONES, M. E. E., LI, H. E., & LIU, J. P. (2007). Potential Roles for Estrogen Regulation of Telomerase Activity in Aging. *Annals of the New York Academy of Sciences, 1114,* 48-55.

Blackburn, E. H. (1991). Structure and function of telomeres. *Nature, 350,* 569-573.

Bonefeld-Jorgensen, E. C., Long, M., Hofmeister, M. V., & Vinggaard, A. M. (2007). Endocrine-Disrupting Potential of Bisphenol A, Bisphenol A Dimethacrylate, 4-n-Nonylphenol, and 4-n-Octylphenol in Vitro: New Data and a Brief Review. *Environmental Health Perspectives, 115,* 69-76.

Bradlow, H. L., Davis, D. L., Lin, G., Sepkovic, D., & Tiwari, R. (1995). Effects of pesticides on the ratio of 16 alpha/2-hydroxyestrone: a biologic marker of breast cancer risk. *Environmental Health Perspectives, 103,* 147-150.

Brede, C., Fjeldal, P., Skjevrak, I., & Herikstad, H. (2003). Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. *Food additives and contaminants, 20,* 684-689.

Brouilette, S., Singh, R. K., Thompson, J. R., Goodall, A. H., & Samani, N. J. (2003). White Cell Telomere Length and Risk of Premature Myocardial Infarction. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23,* 842-846.

Calafat, A. M., Kuklenyik, Z., Reidy, J. A., Caudill, S. P., Ekong, J., & Needham, L. L. (2005). Urinary Concentrations of Bisphenol A and 4-Nonylphenol in a Human Reference Population. *Environmental Health Perspectives, 113,* 391-395.

Calafat, A. M., Ye, X., Wong, L. Y., Reidy, J. A., & Needham, L. L. (2008). Exposure of the U.S. Population to Bisphenol A and 4-tertiary-Octylphenol: 2003GÇô2004. *Environmental Health Perspectives, 116,* 39-44.

Cassidy, A., De Vivo, I., Liu, Y., Han, J., Prescott, J., Hunter, D. J. et al. (2010). Associations between diet, lifestyle factors, and telomere length in women. *The American Journal of Clinical Nutrition, 91,* 1273-1280.

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Research, 30,* e47.

CDC. (2014). http://www.cdc.gov/cancer/breast/.

Ref Type: Online Source

Ceja-Rangel, H. A., S+ínchez-Su+írez, P., Castellanos-Ju+írez, E., Pe+¦aroja-Flores, R., Arenas-Aranda, D. J., Gariglio, P. et al. (2016). Shorter telomeres and high telomerase activity correlate with a highly aggressive phenotype in breast cancer cell lines. *Tumor Biology,* 1-10.

Chin, K., de Solorzano, C. O., Knowles, D., Jones, A., Chou, W., Rodriguez, E. G. et al. (2004). In situ analyses of genome instability in breast cancer. *Nat Genet, 36,* 984-988.

Chitra, K. C., Ramachandra Rao, K., & Mathur, P. P. (2003). Effect of bisphenol A and co-administration of bisphenol A and vitamin C on epididymis of adult rats: A histological and biochemical study. *Asian journal of andrology, 5,* 203-208.

Chou, W. C., Chen, J. L., Lin, C. F., Chen, Y. C., Shih, F. C., & Chuang, C. Y. (2011). Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. *Environmental Health, 10,* 1-10.

Cohn, B., Wolff, M., Cirillo, P., Scholtz, R., Christianson, R., van den Berg, B. et al. (2002). Timing of DDT exposure and breast cancer before age 50. *Epidemiology, 13*.

Coleman, M. P., Quaresma, M., Berrino, F., Lutz, J. M., De Angelis, R., Capocaccia, R. et al. (2008). Cancer survival in five continents: a worldwide population-based study (CONCORD). *The Lancet Oncology, 9,* 730-756.

Collaborative Group on Hormonal Factors in Breast Cancer (1996). Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *The Lancet, 347,* 1713-1727.

Cong, Y. S., Wright, W. E., & Shay, J. W. (2002). Human Telomerase and Its Regulation. *Microbiology and Molecular Biology Reviews, 66,* 407-425.

Dairkee, S. H., Seok, J., Champion, S., Sayeed, A., Mindrinos, M., Xiao, W. et al. (2008). Bisphenol A Induces a Profile of Tumor Aggressiveness in High-Risk Cells from Breast Cancer Patients. *Cancer research, 68,* 2076-2080.

Danaei, G., Vander Hoorn, S., Lopez, A. D., Murray, C. J., & Ezzati, M. Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. *The Lancet, 366,* 1784-1793.

De La Fuente, J. & Dokal, I. (2007). Dyskeratosis congenita: Advances in the understanding of the telomerase defect and the role of stem cell transplantation. *Pediatric Transplantation, 11,* 584-594.

De, V., I, Prescott, J., Wong, J. Y., Kraft, P., Hankinson, S. E., & Hunter, D. J. (2009). A prospective study of relative telomere length and postmenopausal breast cancer risk. *Cancer Epidemiol.Biomarkers Prev., 18,* 1152-1156.

Demierre, A. L., Peter, R., Oberli, A., & Bourqui-Pittet, M. (2012). Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. *Toxicology Letters, 213,* 305-308.

DeSantis, C., Siegel, R., Bandi, P., & Jemal, A. (2011). Breast cancer statistics, 2011. *CA: A Cancer Journal for Clinicians, 61,* 408-418.

Diel, P., Olff, S., Schmidt, S., & Michna, H. (2002). Effects of the environmental estrogens bisphenol A, o,p-DDT, p-tert-octylphenol and coumestrol on

apoptosis induction, cell proliferation and the expression of estrogen sensitive molecular parameters in the human breast cancer cell line MCF-7. *The Journal of Steroid Biochemistry and Molecular Biology, 80,* 61-70.

Donohue, K. M., Miller, R. L., Perzanowski, M. S., Just, A. C., Hoepner, L. A., Arunajadai, S. et al. (2013). Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. *The Journal of allergy and clinical immunology, 131,* 736-742.

Dudinskaya, E. N., Tkacheva, O. N., Shestakova, M. V., Brailova, N. V., Strazhesko, I. D., Akasheva, D. U. et al. (2015). Short telomere length is associated with arterial aging in patients with type 2 diabetes mellitus. *Endocrine Connections, 4,* 136-143.

Duggan, C., Risques, R., Alfano, C., Prunkard, D., Imayama, I., Holte, S. et al. (2014). Change in peripheral blood leukocyte telomere length and mortality in breast cancer survivors. *J.Natl.Cancer Inst., 106,* dju035.

Dunn, B. K., Agurs-Collins, T., Browne, D., Lubet, R., & Johnson, K. A. (2010). Health disparities in breast cancer: biology meets socioeconomic status. *Breast cancer research and treatment, 121,* 281-292.

Dunnwald, L. K., Rossing, M. A., & Li, C. I. (2007). Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. *Breast Cancer Research, 9,* R6.

Durando, M., Kass, L., Piva, J., Sonnenschein, C., Soto, A. M., Luque, E. H. et al. (2007). Prenatal Bisphenol A Exposure Induces Preneoplastic Lesions in the Mammary Gland in Wistar Rats. *Environmental Health Perspectives, 115,* 80-86.

Edginton, A. N. & Ritter, L. (2009). Predicting Plasma Concentrations of Bisphenol A in Children Younger Than 2 Years of Age after Typical Feeding Schedules, using a Physiologically Based Toxicokinetic Model. *Environmental Health Perspectives, 117,* 645-652.

EFSA, E. F. (2015). Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *EFSA Journal, 13*.

Elledge, R. M., Green, S., Pugh, R., Allred, D. C., Clark, G. M., Hill, J. et al. (2000). Estrogen receptor (ER) and progesterone receptor (PgR), by ligand-binding assay compared with ER, PgR and pS2, by immuno-histochemistry in predicting response to tamoxifen in metastatic breast cancer: A Southwest Oncology Group Study. *International Journal of Cancer, 89,* 111-117.

Endogenous Hormones and Breast Cancer Collaborative Group (2011). Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. *Br J Cancer., 105,* 709-722.

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D. et al. (2004). Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences of the United States of America, 101,* 17312-17315.

Falck, F. Jr., Ricci, A. Jr., Wolff, M. S., Godbold, J., & Deckers, P. (1992). Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch Environ Health, 47,* 143-146.

FDA (2014). *Memorandum: 2014 updated safety assessment of bisphenol A (BPA) for use in food contact applications*.

Feng, J. (1995). The RNA component of human telomerase. *Science, 269,* 1236-1241.

Fent, G., Hein, W. J., Moendel, M. J., & Kubiak, R. (2003). Fate of 14C-bisphenol A in soils. *Chemosphere, 51,* 735-746.

Ferlay, J., Soerjomatara, I., Evrik, M., Dikhsit, R., Eser, S., Mathers, C. et al. (2014). GLOBOCAN 2012 v1.1, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. International Agency for Research on Cancer .

Ref Type: Online Source

Fernandez, S. V. & Russo, J. (2010). Estrogen and xenoestrogens in breast cancer. *Toxicol.Pathol., 38,* 110-122.

Fujiwara, M., Okayasu, I., Takemura, T., Tanaka, I., Masuda, R., Furuhata, Y. et al. (2000). Telomerase Activity Significantly Correlates with Chromosome Alterations, Cell Differentiation, and Proliferation in Lung Adenocarcinomas. *Mod Pathol, 13,* 723-729.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y. et al. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. *Journal of Clinical Investigation, 114,* 1752-1761.

Gould, J. C., Leonard, L. S., Maness, S. C., Wagner, B. L., Conner, K., Zacharewski, T. et al. (1998). Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. *Molecular and Cellular Endocrinology, 142,* 203-214.

Gramatges, M. M., Telli, M. L., Balise, R., & Ford, J. M. (2010). Longer relative telomere length in blood from women with sporadic and familial breast cancer compared with healthy controls. *Cancer Epidemiol.Biomarkers Prev., 19,* 605-613.

Greenberg, E. R., Barnes, A. B., Resseguie, L., & Barett, J. A. (1984). Breast cancer in mothers given diethylstilbestrol in pregnancy. *N Engl J Med, 311*.

Griffith, J. D. (1999). Mammalian telomeres end in a large duplex loop. *Cell, 97,* 503-514.

Hanaoka, T., Kawamura, N., Hara, K., & Tsugane, S. (2002). Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidyl ether and mixed organic solvents. *Occupational and Environmental Medicine, 59,* 625-628.

Harley, C. B., Futcher, A. B., & Greider, C. W. (1990a). Telomeres shorten during ageing of human fibroblasts. *Nature, 345,* 458-460.

He, Y., Miao, M., Herrinton, LJ., Wu, C., Yuan, W., & Zhou, Z. (2009). Bisphenol A levels in blood and urine in a Chinese population and the personal factors affecting the levels. *Environmental research, 109,* 629-633.

Hiyama, E. & Hiyama, K. (2007). Telomere and telomerase in stem cells. *British Journal of Cancer, 96,* 1020-1024.

Ho, S. M., Tang, W. Y., Belmonte de Frausto, J., & Prins, G. S. (2006). Developmental Exposure to Estradiol and Bisphenol A Increases Susceptibility to Prostate Carcinogenesis and Epigenetically Regulates Phosphodiesterase Type 4 Variant 4. *Cancer research, 66,* 5624-5632.

Howlader, N., Noone, A. M., Krapcho, M., Neyman, N., Aminou, R., Waldron, W. et al. (2010). *SEER Cancer Statistics Review, 1975-2008* National Cancer Institute.

Hulka, B. S. & Moorman, P. G. (2001). Breast cancer: hormones and other risk factors. *Maturitas, 38,* 103-113.

Hunt, P. A., Koehler, K. E., Susiarjo, M., Hodges, C. A., Ilgan, A., Viogt, R. C. et al. (2003). Bisphenol A exposure causes meiotic aneuploidy in the female mouse. *Curr.Biol., 13,* 546-553.

Ikezuki, Y., Tsutsumi, O., Takai, Y., Kamei, Y., & Taketani, Y. (2002). Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Human Reproduction, 17,* 2839-2841.

Jenkins, S., Wang, J., Eltoum, I., Desmond, R., & Lamartiniere, C. A. (2011). Chronic Oral Exposure to Bisphenol A Results in a Nonmonotonic Dose Response in Mammary Carcinogenesis and Metastasis in MMTV-erbB2 Mice. *Environmental Health Perspectives, 119,* 1604-1609.

Kaletta, T. & Hengartner, M. O. (2006). Finding function in novel targets: C. elegans as a model organism. *Nat Rev Drug Discov, 5,* 387-399.

Kang, H. J. & Kondo, F. (2006). Bisphenol A in the Surface Water and Freshwater Snail Collected from Rivers Around a Secure Landfill. *Bulletin of Environmental Contamination and Toxicology, 76,* 113-118.

Kang, J. H. & Kondo, F. (2002). Bisphenol A Degradation by Bacteria Isolated from River Water. *Archives of Environmental Contamination and Toxicology, 43,* 0265-0269.

Kang, J. H., Ri, N., & Kondo, F. (2004). Streptomyces sp. strain isolated from river water has high bisphenol A degradability. *Letters in Applied Microbiology, 39,* 178-180.

Kang, J. H., Kito, K., & Kondo, F. (8-1-2003). Factors Influencing the Migration of Bisphenol A from Cans. Journal of Food Protection 66[8], 1444-1447.

Ref Type: Abstract

Kang, J. H. & Kondo, F. (2005). Bisphenol A degradation in seawater is different from that in river water. *Chemosphere, 60,* 1288-1292.

Kang, J. H., Kondo, F., & Katayama, Y. (2006). Human exposure to bisphenol A. *Toxicology, 226,* 79-89.

Kawahata, H., Ohta, H., Inoue, M., & Suzuki, A. (2004). Endocrine disrupter nonylphenol and bisphenol A contamination in Okinawa and Ishigaki Islands, JapanGÇôGÇôwithin coral reefs and adjacent river mouths. *Chemosphere, 55,* 1519-1527.

Kawamura, Y., Koyano, Y., Takeda, Y., & Yamada, T. (1998). Migration of bisphenol A from polycarbonate products. *J.Food hyg.Soc.Jpn.,* 206-212.

Kelsey, J. L., Gammon, M. D., & John, E. M. (1993). Reproductive Factors and Breast Cancer. *Epidemiologic Reviews, 15,* 36-47.

Kim, N. W. (1994). Specific association of human telomerase activity with immortal cell and cancer. *Science, 266,* 2011-2015.

Kimura, M., Cherkas, L. F., Kato, B. S., Demissie, S., Hjelmborg, J. B., Brimacombe, M. et al. (2008). Offspring's Leukocyte Telomere Length, Paternal Age, and Telomere Elongation in Sperm. *PLoS Genetics, 4,* e37.

Krieter, D. H., Canaud, B., Lemke, H. D., Rodriguez, A., Morgenroth, A., von Appen, K. et al. (2013). Bisphenol A in Chronic Kidney Disease. *Artificial Organs, 37,* 283-290.

Kuli-ç, A., Plaveti-ç, N. D., Gamulin, S., Jaki-ç-Razumovi-ç, J., Vrbanec, D., & Sirotkovi-ç-Skerlev, M. (2016). Telomerase activity in breast cancer patients: association with poor prognosis and more aggressive phenotype. *Medical Oncology, 33,* 1-6.

Kurebayashi, H., Okudaira, K., & Ohno, Y. (2010). Species difference of metabolic clearance of bisphenol A using cryopreserved hepatocytes from rats, monkeys and humans. *Toxicology Letters, 198,* 210-215.

Kuruto-Niwa, R., Tateoka, Y., Usuki, Y., & Nozawa, R. (2007). Measurement of bisphenol A concentrations in human colostrum. *Chemosphere, 66,* 1160-1164.

Lacey, J. V., Kreimer, A. R., Buys, S. S., Marcus, P. M., Chang, S. C., Leitzmann, M. F. et al. (2009). Breast cancer epidemiology according to recognized breast cancer risk factors in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial Cohort. *BMC Cancer, 9,* 1-8.

Lakind, J.S. & aiman, D.Q. 2008. Bisphenol A (BPA) daily intakes in the United States: Estimates from the 2003–2004 NHANES urinary BPA data. *Journal of Exposure Science and Environmental Epidemiology*, 18, 608-615

LaKind, J. S., Goodman, M., & Naiman, D. Q. (2012). Use of NHANES Data to Link Chemical Exposures to Chronic Diseases: A Cautionary Tale. *PLoS One, 7,* e51086.

LaKind, J. S. & Naiman, D. Q. (2011). Daily intake of bisphenol A and potential sources of exposure: 2005GÇô2006 National Health and Nutrition Examination Survey. *Journal of Exposure Science & Environmental Epidemiology, 21,* 272-279.

Lakkis, N. A., Adib, S. M., Osman, M. H., Musharafieh, U. M., & Hamadeh, G. N. (2010). Breast cancer in Lebanon: Incidence and comparison to regional and Western countries. *Cancer Epidemiology, 34,* 221-225.

Lang, I. A., Galloway, T. S., & Scarlett, A. (2008). ASsociation of urinary bisphenol a concentration with medical disorders and laboratory abnormalities in adults. *JAMA, 300,* 1303-1310.

Lee, J. Y., Jun, N. R., Yoon, D., Shin, C., & Baik, I. (2015). Association between dietary patterns in the remote past and telomere length. *Eur J Clin Nutr, 69,* 1048-1052.

Lee, Y. J., Ryu, H. Y., Kim, H. K., Min, C. S., Lee, J. H., Kim, E. et al. (2008). Maternal and fetal exposure to bisphenol A in Korea. *Reproductive Toxicology, 25,* 413-419.

Levy, T., Agoulnik, I., Atkinson, E. N., Tong, X. W., Gause, H. M., Hasenburg, A. et al. (1998). Telomere length in human white blood cells remains constant with age and is shorter in breast cancer patients. *Anticancer Res., 18,* 1345-1349.

Li, D., Zhou, Z., Qing, D., He, Y., Wu, T., Miao, M. et al. (2010a). Occupational exposure to bisphenol-A (BPA) and the risk of Self-Reported Male Sexual Dysfunction. *Human Reproduction, 25,* 519-527.

Li, D. K., Zhou, Z., Miao, M., He, Y., Qing, D., Wu, T. et al. (2010b). Relationship Between Urine Bisphenol-A Level and Declining Male Sexual Function. *Journal of Andrology, 31,* 500-506.

Lind, P. M. & Lind, L. (2011). Circulating levels of bisphenol A and phthalates are related to carotid atherosclerosis in the elderly. *Atherosclerosis, 218,* 207-213.

Lingner, J., Hughes, T. R., Shevchenko, A., Mann, M., Lundblad, V., & Cech, T. R. (1997). Reverse Transcriptase Motifs in the Catalytic Subunit of Telomerase. *Science, 276,* 561-567.

Linos, E., Willett, W. C., Cho, E., & Frazier, L. (2010). Adolescent diet in relation to breast cancer risk among premenopausal women. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 19,* 689-696.

Maffini, M. V., Rubin, B. S., Sonnenschein, C., & Soto, A. M. (2006). Endocrine disruptors and reproductive health: The case of bisphenol-A. *Molecular and Cellular Endocrinology, 254GÇô255,* 179-186.

Marcand, S., Brevet, V., Mann, C., & Gilson, E. (2000). Cell cycle restriction of telomere elongation. *Current Biology, 10,* 487-490.

Markey, C. M., Coombs, M. A., Sonnenschein, C., & Soto, A. M. (2003). Mammalian development in a changing environment: exposure to endocrine disruptors reveals the development of plasticity of steroid-hormone target organs. *Evol.Dev., 5,* 1-9.

Masutomi, K., Yu, E. Y., Khurts, S., Ben-Porath, I., Currier, J. L., Metz, G. B. et al. (2003). Telomerase Maintains Telomere Structure in Normal Human Cells. *Cell, 114,* 241-253.

Matsumoto, J., Yokota, H., & Yuasa, A. (2002). Developmental increases in rat hepatic microsomal UDP-glucuronosyltransferase activities toward xenoestrogens and decreases during pregnancy. *Environmental Health Perspectives, 110,* 193-196.

McGrath, M., Wong, J. Y. Y., Michaud, D., Hunter, D. J., & De Vivo, I. (2007). Telomere Length, Cigarette Smoking, and Bladder Cancer Risk in Men and Women. *Cancer Epidemiology Biomarkers & Prevention, 16,* 815-819.

Melzer, D., Rice, N. E., Lewis, C., Henley, W. E., & Galloway, T. S. (2010). Association of Urinary Bisphenol A Concentration with Heart Disease: Evidence from NHANES 2003/06. *PLoS One, 5,* e8673.

Mok-Lin, E., Ehrlich, S., Williams, P. L., Petrozza, J., Wright, D. L., Calafat, A. M. et al. (2010). Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. *International Journal of Andrology, 33,* 385-393.

Moon, M. K., Kim, M. J., Jung, I. K., Koo, Y. D., Ann, H. Y., Lee, K. J. et al. (2012). Bisphenol A Impairs Mitochondrial Function in the Liver at Doses below the No Observed Adverse Effect Level. *J Korean Med Sci, 27,* 644-652.

Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saijo, M., Kanamoto, N. et al. (2002). Thyroid hormone action is disrupted by bisphenol A as an antagonist. *The Journal of Clinical Endocrinology & Metabolism, 87,* 5185-5190.

Morla, M., Busquets, X., Pons, J., Sauleda, J., MacNee, W., & Agust+¡, A. G. N. (2006). Telomere shortening in smokers with and without COPD. *European Respiratory Journal, 27,* 525-528.

Mu, J. & Wei, L. X. (2002). Telomere and telomerase in oncology. *Cell Res., 12,* 1-7.

Muller, P., Mayer, S., Mattfeldt, T., Muller, K., Wiegand, P., & Bruderlein, S. (2009). Sex-related differences in length and erosion dynamics of human telomeres favor females. *Aging (Albany NY), 1,* 733-739.

Munoz-de-Toro, M., Markey, C. M., Wadia, P. R., Luque, E. H., Rubin, B. S., Sonnenschein, C. et al. (2005). Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. *Endocrinology, 146,* 4138-4147.

Murray, T. J., Maffini, M. V., Ucci, A. A., Sonnenschein, C., & Soto, A. M. (2007). Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. *Reprod.Toxicol., 23,* 383-390.

NIWA, T., FUJIMOTO, M., KISHIMOTO, K., YABUSAKI, Y., ISHIBASHI, F., & KATAGIRI, M. (2001). Metabolism and Interaction of Bisphenol A in Human Hepatic Cytochrome P450 and Steroidogenic CYP17. *Biological and Pharmaceutical Bulletin, 24,* 1064-1067.

Odagiri, E., Kanda, N., Jibiki, K., Demura, R., Aikawa, E., & Demura, H. (1994). Reduction of telomeric length and c-erbb-2 gene amplification in human breast

cancer, fibroadenoma, and gynecomastia. Relationship to histologic grade and clinical parameters. *Cancer, 73,* 2978-2984.

Onitilo, A. A., Engel, J. M., Greenlee, R. T., & Mukesh, B. N. (2009). Breast Cancer Subtypes Based on ER/PR and Her2 Expression: Comparison of Clinicopathologic Features and Survival. *Clinical Medicine & Research, 7,* 4-13.

Padmanabhan, V., Siefert, K., Ransom, S., Johnson, T., Pinkerton, J., Anderson, L. et al. (2008). Maternal bisphenol-A levels at delivery: a looming problem? *Journal of perinatology : official journal of the California Perinatal Association, 28,* 258-263.

Palm, W. & de Lange, T. (2008). How Shelterin Protects Mammalian Telomeres. *Annual Review of Genetics, 42,* 301-334.

Palmer, J. R., Boggs, D. A., Wise, L. A., Ambrosone, C. B., Adams-Campbell, L. L., & Rosenberg, L. (2011). Parity and lactation in relation to estrogen receptor negative breast cancer in African American women. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 20,* 1883-1891.

Pavanello, S., Hoxha, M., Dioni, L., Bertazzi, P. A., Snenghi, R., Nalesso, A. et al. (2011). Shortened telomeres in individuals with abuse in alcohol consumption. *International Journal of Cancer, 129,* 983-992.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RTGÇôPCR. *Nucleic Acids Research, 29,* e45.

Pfeifer, D., Chung, Y. M., & Hu, M. C. (2015). Effects of low-dose bisphenol A on DNA damage and proliferation of breast cells: the role of c-Myc. *Environmental Health Perspectives, 123,* 1271-1279.

Poimenova, A., Markaki, E., Rahiotis, C., & Kitraki, E. (2010). Corticosterone-regulated actions in the rat brain are affected by perinatal exposure to low dose of bisphenol A. *Neuroscience, 167,* 741-749.

Radpour, R., Barekati, Z., Haghighi, M. M., Kohler, C., Asadollahi, R., Torbati, P. M. et al. (2010). Correlation of telomere length shortening with promoter methylation profile of p16/Rb and p53/p21 pathways in breast cancer. *Mod Pathol, 23,* 763-772.

Raynaud, C. M., Hernandez, J., Llorca, F. d. r. P., Nuciforo, P., Mathieu, M. C., Commo, F. et al. (2010). DNA Damage Repair and Telomere Length in Normal Breast, Preneoplastic Lesions, and Invasive Cancer. *American Journal of Clinical Oncology, 33*.

Reddel, R. R. (2003). Alternative lengthening of telomeres, telomerase, and cancer. *Cancer Letters, 194,* 155-162.

Rees Clayton, E. M., Todd, M., Dowd, J. B., & Aiello, A. E. (2011). The Impact of Bisphenol A and Triclosan on Immune Parameters in the U.S. Population, NHANES 2003GÇô2006. *Environmental Health Perspectives, 119,* 390-396.

Roger, P., Sahla, M. E., M+ñkel+ñ, S., Gustafsson, J. +., Baldet, P., & Rochefort, H. (2001). Decreased Expression of Estrogen Receptor +¦ Protein in Proliferative Preinvasive Mammary Tumors. *Cancer research, 61,* 2537-2541.

Routledge, E. J., White, R., Parker, M. G., & Sumpter, J. P. (2000). Differential Effects of Xenoestrogens on Coactivator Recruitment by Estrogen Receptor (ER) +¦ and ER+¦. *Journal of Biological Chemistry, 275,* 35986-35993.

Roy, D., Colerangle, J. B., & Singletary, K. W. (1998). Is Exposure To Environmental Or Industrial Endocrine Disrupting Estrogen-Like Chemicals Able To Cause GenomicInstability?*Frontiers in Bioscience, 3,* 913-921.

Roy, D., Palangat, M., Chen , C. W., Thomas, R. D., Colerangle, J., Atkinson, A. et al. (1997). Biochemical and molecular changes at the cellular level in response to exposure to environmental estrogen-like chemicals. *Journal of toxicology and environmental health, 50,* 1-29.

Rubin, B. S., Murray, M. K., Damassa, D. A., Kings, J. C., & Soto, A. M. (2001). Perinatal exposure to low doses of bisphenol-A affects body weight, patterns of estrous cyclicity and plasma LH levels. *Environmental Health Perspectives, 109,* 675-680.

Rubin, B. S. (2011). Bisphenol A: An endocrine disruptor with widespread exposure and multiple effects. *The Journal of Steroid Biochemistry and Molecular Biology, 127,* 27-34.

Rubtsova, M. P., Vasilkova, D. P., Malyavko, A. N., Naraikina, Y., Zvereva, M. I., & Dontsova, O. A. (2012). Telomere Lengthening and Other Functions of TelomeraseGÇ¬. *Acta Naturae, 4,* 44-61.

Samassekou, O., Gadji, M., Drouin, R. +., & Yan, J. (2010). Sizing the ends: Normal length of human telomeres. *Annals of Anatomy - Anatomischer Anzeiger, 192,* 284-291.

Sampson, M. J., Winterbone, M. S., Hughes, J. C., Dozio, N., & Hughes, D. A. (2006). Monocyte Telomere Shortening and Oxidative DNA Damage in Type 2 Diabetes. *Diabetes Care, 29,* 283-289.

Sargis, R. M., Johnson, D. N., Choudhury, R. A., & Brady, M. J. (2010). Environmental Endocrine Disruptors Promote Adipogenesis in the 3T3-L1 Cell Line through Glucocorticoid Receptor Activation. *Obesity (Silver Spring, Md.), 18,* 1283-1288.

Sasaki, N., Okuda, K., Kato, T., Kakishima, H., Okuma, H., Abe, K. et al. (2005). Salivary bisphenol-A levels detected by ELISA after restoration with composite resin. *Journal of Materials Science: Materials in Medicine, 16,* 297-300.

Schonfelder, G., Wittfoht, W., Hopp, H., Talsness, C. E., Paul, M., & Chahoud, I. (2002). Parent bisphenol A accumulation in the human maternal-fetal-placental unit. *Environmental Health Perspectives, 110,* A703-A707.

Shammas, M. A. (2011). Telomeres, lifestyle, cancer, and aging. *Current Opinion in Clinical Nutrition and Metabolic Care, 14,* 28-34.

Shamseddine, A., Saleh, A., Charafeddine, M., Seoud, M., Mukherji, D., Temraz, S. et al. (2014). Cancer trends in Lebanon: a review of incidence rates for the period of 2003GÇô2008 and projections until 2018. *Population Health Metrics, 12,* 4.

Shankar, A. & Teppala, S. (2012). Urinary Bisphenol A and Hypertension in a Multiethnic Sample of US Adults. *Journal of Environmental and Public Health, 2012*.

Shankar, A. & Teppala, S. (2011). Relationship between Urinary Bisphenol A Levels and Diabetes Mellitus. *The Journal of Clinical Endocrinology and Metabolism, 96,* 3822-3826.

Shay, J. W. & Wright, W. E. (2011). Role of telomeres and telomerase in cancer. *Seminars in Cancer Biology, 21,* 349-353.

Shay, J. W. (1997). Telomerase in human development and cancer. *Journal of Cellular Physiology, 173,* 266-270.

Shen, J., Gammon, M. D., Terry, M. B., Wang, Q., Bradshaw, P., Teitelbaum, S. L. et al. (2009). Telomere length, oxidative damage, antioxidants and breast cancer risk. *International Journal of Cancer, 124,* 1637-1643.

Singletary, K. W. & Gapstur, S. M. (2001). Alcohol and breast cancer: Review of epidemiologic and experimental evidence and potential mechanisms. *JAMA, 286,* 2143-2151.

Song, Y., You, N. C., Song, Y., Kang, M. K., Hou, L., Wallace, R. et al. (2013). Intake of Small-to-Medium-Chain Saturated Fatty Acids Is Associated with Peripheral Leukocyte Telomere Length in Postmenopausal Women. *The Journal of Nutrition, 143,* 907-914.

Sprague, B. L., Trentham-Dietz, A., Hedman, C. J., Wang, J., Hemming, J. D., Hampton, J. M. et al. (2013). Circulating serum xenoestrogens and mammographic breast density. *Breast Cancer Research, 15,* R45.

Stendahl, M., Kronblad, +., Ryd+¬n, L., Emdin, S., Bengtsson, N. O., & Landberg, G. (2004). Cyclin D1 overexpression is a negative predictive factor for tamoxifen response in postmenopausal breast cancer patients. *British Journal of Cancer, 90,* 1942-1948.

Stuart-Harris, R., Caldas, C., Pinder, S. E., & Pharoah, P. (2008). Proliferation markers and survival in early breast cancer: A systematic review and meta-analysis of 85 studies in 32,825 patients. *The Breast, 17,* 323-334.

Sugiura-Ogasawara, M., Ozaki, Y., Sonta, S. I., Makino, T., & Suzumori, K. (2005). Exposure to bisphenol A is associated with recurrent miscarriage. *Human Reproduction, 20,* 2325-2329.

Sun, Q., Cornelis, M. C., Townsend, M. K., Tobias, D. K., Eliassen, A. H., Franke, A. A. et al. (2014). Association of Urinary Concentrations of Bisphenol A and Phthalate Metabolites with Risk of Type 2 Diabetes: A Prospective Investigation in the NursesGÇÖ Health Study (NHS) and NHSII Cohorts. *Environmental Health Perspectives, 122,* 616-623.

Sun, Y., Irie, M., Kishikawa, N., Wada, M., Kuroda, N., & Nakashima, K. (2004). Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. *Biomedical Chromatography, 18,* 501-507.

Svenson, U., Nordfj+ñll, K., Stegmayr, B., Manjer, J., Nilsson, P., Tavelin, B. r. et al. (2008). Breast Cancer Survival Is Associated with Telomere Length in Peripheral Blood Cells. *Cancer research, 68,* 3618-3623.

Tahara, H., Nakanishi, T., Kitamoto, M., Nakashio, R., Shay, J. W., Tahara, E. et al. (1995). Telomerase Activity in Human Liver Tissues: Comparison between Chronic Liver Disease and Hepatocellular Carcinomas. *Cancer research, 55,* 2734-2736.

Takao, Y., Lee, H. C., Ishibashi, Y., Kohra, S., Tominaga, N., & Arizono, K. (1999). Fast screening method for bisphenol A in environmental water and in food by solid-phase microextraction (SPEM). *J.Health Sci., 45,* 39.

Takayanagi, S., Tokunaga, T., Liu, X., Okada, H., Matsushima, A., & Shimohigashi, Y. (2006). Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor +¦ (ERR+¦) with high constitutive activity. *Toxicology Letters, 167,* 95-105.

Takubo, K., Izumiyama-Shimomura, N., Honma, N., Sawabe, M., Arai, T., Kato, M. et al. (2002). Telomere lengths are characteristic in each human individual. *Experimental Gerontology, 37,* 523-531.

Teeguarden, J. G., Waechter, J. M., Clewell, H. J., Covington, T. R., & Barton, H. A. (2005). Evaluation of Oral and Intravenous Route Pharmacokinetics, Plasma Protein Binding, and Uterine Tissue Dose Metrics of Bisphenol A: A Physiologically Based Pharmacokinetic Approach. *Toxicological Sciences, 85,* 823-838.

Teixeira, M. T., Arneric, M., Sperisen, P., & Lingner, J. (2004). Telomere Length Homeostasis Is Achieved via a Switch between Telomerase- Extendible and -Nonextendible States. *Cell, 117,* 323-335.

Teng, C., Goodwin, B., Shockley, K., Xia, M., Huang, R., Norris, J. et al. (2013). Bisphenol A affects androgen receptor function via multiple mechanisms. *Chemico-Biological Interactions, 203,* 556-564.

Thayer, K. A., Doerge, D. R., Hunt, D., Schurman, S. H., Twaddle, N. C., Churchwell, M. I. et al. (2015). Pharmacokinetics of bisphenol A in humans following a single oral administration. *Environment International, 83,* 107-115.

Thomas, P. & Dong, J. (2006). Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: A potential novel mechanism of endocrine disruption. *The Journal of Steroid Biochemistry and Molecular Biology, 102,* 175-179.

Trasande, L., Attina, T. M., & Blustein, J. (2012). ASsociation between urinary bisphenol a concentration and obesity prevalence in children and adolescents. *JAMA, 308,* 1113-1121.

Trdan Lusin, T., Ro+íkar, R., & Mrhar, A. (2012). Evaluation of bisphenol A glucuronidation according to UGT1A1*28 polymorphism by a new LCGÇôMS/MS assay. *Toxicology, 292,* 33-41.

Tyl, R. W., Myers, C. B., Marr, M. C., Thomas, B. F., Keimowitz, A. R., Brine, D. R. et al. (2002). Three-Generation Reproductive Toxicity Study of Dietary Bisphenol A in CD Sprague-Dawley Rats. *Toxicological Sciences, 68,* 121-146.

Valdes, A. M., Andrew, T., Gardner, J. P., Kimura, M., Oelsner, E., Cherkas, L. F. et al. Obesity, cigarette smoking, and telomere length in women. *The Lancet, 366,* 662-664.

Valdes, A. M., Richards, J. B., Gardner, J. P., Swaminathan, R., Kimura, M., Xiaobin, L. et al. (2007). Telomere length in leukocytes correlates with bone mineral density and is shorter in women with osteoporosis. *Osteoporosis International, 18,* 1203-1210.

van der Harst, P., van der Steege, G., de Boer, R. A., Voors, A. A., Hall, A. S., Mulder, M. J. et al. (2007). Telomere Length of Circulating Leukocytes Is Decreased in Patients With Chronic Heart Failure. *Journal of the American College of Cardiology, 49,* 1459-1464.

Vandenberg, L. N., Schaeberle, C. M., Rubin, B. S., Sonnenschein, C., & Soto, A. M. (2013). The male mammary gland: a target for the xenoestrogen bisphenol A. *Reprod.Toxicol., 37,* 15-23.

Vandenberg, L. N., Chahoud, I., Heindel, J. J., Padmanabhan, V., Paumgartten, F. J., & Schoenfelder, G. (2010). Urinary, Circulating, and Tissue Biomonitoring Studies Indicate Widespread Exposure to Bisphenol A. *Environmental Health Perspectives, 118,* 1055-1070.

Vandenberg, L. N., Maffini, M. V., Sonnenschein, C., Rubin, B. S., & Soto, A. M. (2009). Bisphenol-A and the Great Divide: A Review of Controversies in the Field of Endocrine Disruption. *Endocrine Reviews, 30,* 75-95.

Vanitha, M. K., Sakthisekaran, D., & Anandakumar, P. (2014). BREAST CANCER: TYPES, EPIDEMIOLOGY & AETIOLOGY - A

REVIEW. *Adv J Pharm Life sci Res, 2,* 29-38.

Vaziri, H., Dragowska, W., Allsopp, R. C., Thomas, T. E., Harley, C. B., & Lansdorp, P. M. (1994). Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proceedings of the National Academy of Sciences of the United States of America, 91,* 9857-9860.

Vogel, S. A. (2009). The Politics of Plastics: The Making and Unmaking of Bisphenol A GÇ£SafetyGÇ¥. *American Journal of Public Health, 99,* S559-S566.

vom Saal, F. S., Cooke, P. S., Buchanan, D. L., Palanza, P., Thayer, K. A., Nagel, S. C. et al. (1998). A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicol.Ind.Health, 14,* 239-260.

Vorherr, H. (1974). The breast: morphology, physiology, and lactation. *New York: Academic Press.*.

Wade, C. B., Robinson, S., Shapiro, R. A., & Dorsa, D. M. (2001). Estrogen Receptor (ER)+¦ and ER+¦ Exhibit Unique Pharmacologic Properties When Coupled to

Activation of the Mitogen-Activated Protein Kinase Pathway. *Endocrinology, 142,* 2336-2342.

Wang, T., Li, M., Chen, B., Xu, M., Xu, Y., Huang, Y. et al. (2011). Urinary Bisphenol A (BPA) Concentration Associates with Obesity and Insulin Resistance. *The Journal of Clinical Endocrinology & Metabolism, 97,* E223-E227.

Watson, C. S., Bulayeva, N. N., Wozniak, A. L., & Alyea, R. A. (2007). Xenoestrogens are potent activators of nongenomic estrogenic responses. *Steroids, 72,* 124-134.

Weigel, M. T. & Dowsett, M. (2010). Current and emerging biomarkers in breast cancer: prognosis and prediction. *Endocrine-Related Cancer, 17,* R245-R262.

Weischer, M., Bojesen, S. E., & Nordestgaard, B. r. G. (2014). Telomere Shortening Unrelated to Smoking, Body Weight, Physical Activity, and Alcohol Intake: 4,576 General Population Individuals with Repeat Measurements 10 Years Apart. *PLoS Genetics, 10,* e1004191.

Welshons, W. V., Nagel, S. C., & Saal, F. S. v. (2006). Large Effects from Small Exposures. III. Endocrine Mechanisms Mediating Effects of Bisphenol A at Levels of Human Exposure. *Endocrinology, 147,* S56-S69.

Werner, C., F++rster, T., Widmann, T., P+¦ss, J., Roggia, C., Hanhoun, M. et al. (2009). Physical Exercise Prevents Cellular Senescence in Circulating Leukocytes and in the Vessel Wall. *Circulation, 120,* 2438-2447.

WHO (2014). *Cancer country profile*.

Wu, X., Amos, C. I., Zhu, Y., Zhao, H., Grossman, B. H., Shay, J. W. et al. (2003). Telomere Dysfunction: A Potential Cancer Predisposition Factor. *Journal of the National Cancer Institute, 95,* 1211-1218.

Yang, M., Ryu, J. H., Jeon, R., Kang, D., & Yoo, K. Y. (2008). Effects of bisphenol A on breast cancer and its risk factors. *Archives of Toxicology, 83,* 281-285.

Yang, X., Doerge, D. R., Teeguarden, J. G., & Fisher, J. W. (2015). Development of a physiologically based pharmacokinetic model for assessment of human exposure to bisphenol A. *Toxicology and Applied Pharmacology, 289,* 442-456.

Yang, Y. J., Hong, Y. C., Oh, S. Y., Park, M. S., Kim, H., Leem, J. H. et al. (2009). Bisphenol A exposure is associated with oxidative stress and inflammation in postmenopausal women. *Environmental research, 109,* 797-801.

Ye, X., Kuklenyik, Z., Needham, L. L., & Calafat, A. M. (2006). Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching-high performance liquid chromatography-isotope dilution tandem mass spectrometry. *Journal of Chromatography B, 831,* 110-115.

Yoshino, S., Yamaki, K., Li, X., Sai, T., Yanagisawa, R., Takano, H. et al. (2004). Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. *Immunology, 112,* 489-495.

Zafra, A., Olmo, M., Pulgar, R., Naval+¦n, A., & V+¡lchez, J. L. (2002). Determination of bisphenol-a and related compounds in human saliva by gas chromatography mass spectrometry. *Chromatographia, 56,* 213-218.

Zgheib, N. K., Shamseddine, A. A., Geryess, E., Tfayli, A., Bazarbachi, A., Salem, Z. et al. (2013). Genetic polymorphisms of CYP2E1, GST, and NAT2 enzymes are not associated with risk of breast cancer in a sample of Lebanese women. *Mutat.Res., 747-748,* 40-47.

Zhao, Y., Sfeir, A. J., Zou, Y., Buseman, C. M., Chow, T. T., Shay, J. W. et al. (2009). Telomere extension occurs at most chromosome ends and is uncoupled from fill-in in human cancer cells. *Cell, 138,* 463-475.

Zheng, Y. L., Ambrosone, C., Byrne, C., Davis, W., Nesline, M., & McCann, S. E. (2010). Telomere length in blood cells and breast cancer risk: investigations in two case-control studies. *Breast Cancer Res.Treat., 120,* 769-775.

Zheng, Y. L., Zhou, X., Loffredo, C. A., Shields, P. G., & Sun, B. (2011). Telomere deficiencies on chromosomes 9p, 15p, 15q and Xp: potential biomarkers for breast cancer risk. *Human Molecular Genetics, 20,* 378-386.