Arch. Bas. App. Med. 2 (2014) 87 - 91

ARCHIVES OF BASIC & APPLIED MEDICINE

Research Article

Serum Polychlorinated Biphenyls and Bisphenol-A Levels in Nigerian Women with Breast Cancer

Ajayi O.O*[1], Charles-Davies M.A[1], Anetor J.I[1], Ademola A.F[2]

[1]*Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria*

[2]*Division of Surgical Oncology, Department of Surgery, University College Hospital, Ibadan, Nigeria.*

Accepted in final form: 26th June 2014

Abstract

Endocrine disruptors (EDs)-polychlorinated biphenyls (PCBs) and bisphenol-A (BPA) have been associated with increased risk of breast cancer (B.Ca). In Nigeria, there is increase in breast cancer incidence. This has been attributed to increased use and exposure to EDs. Although, several studies have been done on breast cancer, there is paucity of information on the serum levels of these EDs in Nigerian women. This study therefore aims at determining the serum levels of PCBs and BPA in breast cancer patients.Eighty women aged 18-70 years were recruited with informed consent after institutional ethical approval. They were 40 participants with breast cancer pre-treatment (cases) matched with 40 apparently healthy women (controls). They were recruited from a tertiary hospital in Ibadan, Nigeria. Demographic indices: age, age at menarche (AM), age at menopause (AMP) and anthropometric indices: height, weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR) and waist-height ratio (WHT) were obtained from the pre-test questionnaire administered and standard methods respectively.10 ml of venous blood was collected from each participant and sera obtained were analyzed for PCB and BPA. These were determined by High performance liquid chromatography (HPLC). Data were analyzed using Student t-test and Pearson correlation coefficient, p was significant at <0.05.Results showed increases in the height, weight, WC, HC, WHR, WHT, PCB and BPA serum levels in cases compared with controls. Positive correlations were observed among WC and WHR, WHT, HC; HC and WHT; WHR and WHT in both cases and controls. However, positive correlations among height, weight, WC and WHR; BMI and weight were observed in cases only. Weight correlated negatively with age at menarche (AM) only in controls. The differences and correlations were statistically significant ($p<0.05$).Mechanisms involving PCB, BPA and adiposity may be involved in breast cancer aetiology. Reduction of environmental pollution and dietary modulation may be helpful.

.

Keywords:Breast cancer, bisphenol-A, polychlorinated biphenyls, adiposity, age at menopause

INTRODUCTION

Breast cancer is one of the most common types of cancer and affects millions of women around the world with a noticeable fatality rate (Msolly *et al.*, 2011). In Nigeria, the incidence of breast cancer has been reported to be on the increase (Adesunkanmi *et al.*, 2006). This increase in incidence rate has been attributed to changes in demography, socio-economic status and epidemiological risk factors. Mortality rates are on the decline in the developed countries (America, Australia and Western Europe) due to early diagnosis, screening and improved cancer treatment programs. Conversely, mortality rates are on the increase in the developing countries as well as in Eastern and Central Europe (Adesunkanmi *et al.*, 2006).

Body mass index (BMI) has been reported as a measure of overall adiposity, while waist circumference (WC) and waist-hip-ratio (WHR) are reliable proxy measures of abdominal fat (Chakraborty and Bose, 2009).Therefore, they could be used to identify overweight and obesity. However, the waist circumference alone is a somewhat better indicator of visceral adiposity than the waist to hip ratio (Donohoe *et al*., 2011). These measures of adiposity have been widely recommended for epidemiological surveys because of their independent association with major non-communicable metabolic diseases including breast cancer (Chakraborty and Bose, 2009). The relationships between adiposity and menopausal status coupled with prognosis of breast cancer have been the subject of considerable research for the past 40 years or more (Renehan *et al.*, 2008).

The adipose tissue is a heterogenous mixture of adipocytes, stromal pre-adipocytes, immune cells and endothelium (Halberg *et al*., 2008). It functions as a complex endocrine organ secreting a host of factors collectively referred to as adipokines (Halberg *et al.*, 2008). Moreover, the adipose tissue vascularisation functions in a delicate balance in tissue homeostasis, the perturbation initiated by tumour growth dysregulate all involved cell types (Haighton *et al.*, 2002). This is seen in breast cancer in which transformed ductal epithelial cell break through the basal lamina and invade the mammary stromal compartment which is highly enriched in adipose tissue after filling the lumen of mammary duct (Haighton *et al.*, 2002).

Human exposure to some environmental chemicals described as endocrine disruptors has been shown to alter endocrine functions resulting in adverse physiologic functions. These endocrine disruptors include-polychlorinated bisphenyls (PCBs) and bisphenol-A (BPA). The most relevant route of exposure is oral (Brody *et al*, 2007).

**Author for correspondence - E-mail:* olufema@gmail.com

Endocrine disruptors mimic the action of oestrogens binding to the receptor, move into the nucleus and disrupt cell growth and division leading to uncontrolled cell proliferation. It has been reported that some of these toxicants increase the risk of breast cancer and its progression (Brophy *et al*., 2012).

Bisphenol-A is a momomer of plastic materials that are widely used in daily life. BPA is used for the production of epoxy resins, phenol resins, polycarbonates, polyacrylates, polyesters and lacquer coatings on food cans. Hence, there is widespread human exposure (Brody *et al*, 2007; Betancourt*et al*., 2012).

Human exposure to bisphenol-A of up to 50ug/dl/day is considered safe by United States Environmental Protection Agency (USEPA). Currently, the safety of BPA products is controversial. Some studies have demonstrated the ability of BPA to weakly stimulate oestrogen receptors (Fernandez and Russo, 2010). BPA binds with higher affinity to oestrogen receptor beta (ERβ) which is expressed in almost every tissue in the body (Brisken, 2008). High levels of these endocrine disruptors have been suggested in the serum breast cancer patients (Calafat *et al*., 2013).

Polychlorinated biphenyls are members of a chemical family that were widely used in the past in industry as lubricants, coatings and insulation materials for dielectric equipment like transformers and capacitors (Iyengar, 2005). PCBs were also used in common consumer items such as hydraulic fluid, fluorescent lights, televisions and other appliances. The release of PCBs to the environment has been reported to be through poorly maintained hazardous waste dumps and city landfills, illegal or improper dumping of hydraulic fluids/coolants, leaks from electrical transformers and other equipment, burning of medical, industrial or city waste from older consumer goods like televisions (Gray *et al*, 2009). However, there is paucity of information on the serum level of PCBs in non-occupationally exposed women in Nigeria. This study estimated the serum levels of PCBs and BPA in Nigerian breast cancer patients.

MATERIALS AND METHODS

Study design and duration:

This case-control study was conducted after ethical approval (UI/EC/10/0193) was obtained from the joint ethical committee of University of Ibadan/University College Hospital, Ibadan, Nigeria.

Subjects:

A total of 80 women aged 18-70 years were recruited for this study after informed consent. These were 40 breast cancer patients who have not commenced treatment (cases) and 40 apparently healthy women (controls) were age-matched with the cases. Subjects with other types of cancer, diabetes mellitus, hypertension, pregnancy, lactating mothers and subjects on hormonal therapy were excluded from the study. Moreover, breast cancer patients who had commenced treatment were excluded. The cases were recruited by surgical oncologist at the Surgical Oncology Division, Department of Surgery, University College Hospital (UCH), Ibadan, Nigeria.

Controls:

These were apparently healthy individuals without breast cancer. They were randomly recruited within Ibadan city. Fasting blood glucose was determined to exclude type 2 diabetes mellitus.

Demographic and Anthropometric Indices

Demographic indices: Age, age at menarche and age at menopause were obtained through questionnaires administered to the subjects.

Anthropometric indices: weight, height, body mass index, waist circumference, hip circumference, waist-hip ratio, waist-height ratio were measured.

Weight

This was taken with a bathroom weighing scale placed on a flat surface. The subjects while wearing light clothing and without any shoes on were made to stand on the scale with the indicator at zero. The reading was recorded to the nearest 0.5kg.

Height

This was measured against a flat, vertical surface with the subjects standing bare footed in an upright position without any head gear on. Without raising the heels from the ground and the feet kept together. Measurements were taken with a sliding headpiece brought to the vertex of the subject's head. The reading at this level was taken to the nearest 0.1m.

Body Mass Index (BMI): This was calculated from the body weight and height of the subjects using the formula stated below.

$$BMI\ (Kg/m^2) = weight\ (kg)/height\ (m^2).$$

Waist and hip circumferences: Waist circumference (in cm) was measured using a measuring tape placed at the navel level while hip circumference (in cm) was measured at the widest circumference of the hip over light clothing using a non-stretchable measuring tape without any pressure on the body surface. Both indices were recorded to the nearest 0.1cm.

Waist-hip ratio

This was calculated from the ratio of the waist circumference to the hip circumference i.e. waist/height.

Waist-height ratio

This was calculated from the ratio of the waist circumference to the height.

Sample Collection

Ten (10) ml of venous blood was obtained from antecubital fossa vein of each subject. The blood sample was allowed to clot, retracted and centrifuged at 3500 rpm for 5 minutes after which serum was separated and stored at -20^{o}C until analysis was done.

Biochemical Investigations

The biochemical indices estimated were serum polychlorinated biphenyls (PCBs) and bisphenol A (BPA). Serum PCBs and BPA were estimated by high performance liquid chromatography (ALLIANCE, e2695; Waters, USA).

Analytical Method for BPA

Serum samples were fortified with 12.5 nanograms of isotopically labeled phthalate metabolites, 50 nanograms of labelled bisphenol-A, 250 nanograms of 4-methylumbelliferone glucuronide, 300 microlitres of ammonium acetate buffer (PH 6.5) and 10 microliters of β-glucuronidase (Escherichia coli K12, Roche Biomedical). The samples were mixed and incubated at 37^{o}C overnight to allow for the deglucuronidation.

Following enzymatic hydrolysis, a 20µL aliquot of the sample was added to 70µL of HPLC- grade water and 10ng of labeled 4-methylumbelliferone to determine deglucuronidation efficiency. The remaining sample was loaded on to Zymark rapid trace solution for automated solid phase extraction (SPE). The 60milligram/3mL Oasis-HLB cartridges were conditioned with HPLC-grade methanol (2ml) and 0.1 M formic acid (2mL). The samples were diluted with 5 mL of 0.1 M formic acid and loaded on the SPE cartridge at a rate of 1.0mL/min. The cartridge was washed with water (1mL) and 10% methanol in water (2mL) at a flow rate of 1mL/min. The samples were eluted with 1.0mL of acetonitrile at a flow rate of 0.5 mL/min. The eluate was evaporated to dryness under a stream of dry nitrogen and the residue was resuspended in 85% methanol in water (200 microliters) and transferred to glass autosampler vials. Quality control of the analysis was maintained by analyzing a method blank (calf serum) and two spiked calf serum samples (20ng/mL). The detection limit (0.2ng/mL) was based upon a lower calibration standard (0.5ng/ml) which gave an instrument signal to noise response of 3:1.

Analytical Method for PCBs: Serum samples were mixed with methanol and a mixture of internal standards were added to correct for recovery and ensure quality control. The samples were then extracted three times with n-hexane-diethyl-ether (1:1 v/v). After evaporation of the solvents the fat content was determined gravimetrically. The fat was redissolved in n-hexane and treated with concentrated sulphuric acid. The PCBs were separated from the bulk of the chlorinated compounds by elution through a silica gel column (4.5g of 3% water-deactivated silica-gel). The first fraction, containing the PCBs was eluted with 30ml of n-hexane. The columns were of different polarity to ease identification of analytes which was based on retention times relative to internal standards. Quantification was performed using multilevel calibration curves obtained by injection of standard solutions of at least three different concentrations. The limit of determination (LOD) was determined as three standard deviations (SD) above the value of the blank and varied between 1 and 7 pg/g serum (not lipid adjusted). Samples with concentrations of LODs three SD above the blank have a 99% probability of being non-zero. To increase this probability, the quantification limits (LOQ) were set at higher levels than the LODs. In this case the lowest standard concentration was used; 10pg/g serum. The reproducibility of the method was demonstrated by 21 replicate determinations using an in-house control serum sample included in the analytical batches during the course of the study.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) software version 17.00 computer software was used for the analysis of data. Two-tailed independent t-test of significance at 95% confidence limit with $p<0.05$ were considered significant for the variables. Pearson correlation coefficient was used to find relationships between quantitative variables.

RESULTS

Table 1 compares mean±SE of age, age at menarche(AM), age at menopause(AMP), weight, height, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), waist-height ratio (WHR), serum polychlorinated biphenyls and bisphenol-A in breast cancer subjects(cases) and controls. Comparison showed significant differences ($p<0.05$) in mean±SE of weight, height, waist circumference, hip circumference, waist-hip ratio, waist-height ratio, polychlorinated biphenyls and bisphenol-A in breast cancer subjects and controls. There were no significant differences in age, age at menarche, age at menopause and body mass index.

Table1.

Comparison of demographic, anthropometric and biochemical indices breast cancer subjects and controls using Student's t-test.

PARAMETERS	**CASES**		**CONTROL**			
Demographic Indices	**N**	**Mean±S.E**	**N**	**Mean±S.E**	**t**	**p**
Age (years)	40	47.90±1.83	40	51.10±2.32	-1.081	0.283
Age at Menarche (years)	39	15.23±0.31	40	14.53±0.35	1.508	0.136
Age at Menopause(years)	12	46.75±1.53	18	49.11±0.66	-1.595	0.122
Anthropometric Indices						
Weight(Kg)	39	69.62±2.41	40	58.89±1.61	3.715	0.000*****
Height(m)	39	1.65±0.01	40	1.56±0.01	5.439	0.000*****
BMI (kg/m^2)	39	25.65±0.84	40	23.82±0.64	1.742	0.085
Waist Circumference (cm)	39	86.54±1.55	40	76.65±1.45	4.665	0.000*****
Hip Circumference (cm)	39	99.00±1.44	40	94.02±1.24	2.620	0.011*****
Waist:Hip	39	0.87±0.01	40	0.82±0.01	4.075	0.000*****
Waist:Height	38	52.52±0.90	40	48.98±0.89	2.795	0.007*****
Biochemical Parameters						
PCB(µg/dl)	40	0.81±0.67	40	0.35±0.01	6.805	0.000*****
BPA (mg/dl)	40	0.79±0.46	40	0.299±0.01	10.115	0.000*****

*Values are mean±SE; *****=significant, SE= standard error, n= number of subjects, p=probabality, PCB=polychlorinated Biphenyls, BPA=bisphenol-A, BMI=body mass index*

Table 2.
Correlation of demographic with anthropometric indices in females with breast cancer and controls.

Indices	Breast cancer n=40 (r,p-values)	Controls n=40 (r,p-values)
Height vs Weight	0.349, 0.029*	0.139, 0.391
Height vs WC	0.360, 0.026*	0.222, 0.169
Height vs WHR	0.354, 0.029*	0.204, 0.207
Weight vs BMI	0.925, 0.000*	0.197, 0.224
Weight vs AM	0.021, 0.899	-0.328, 0.039*
WC vs WHR	0.595, 0.000*	0.697, 0.000*
WC vs WHT	0.930, 0.000*	0.937, 0.000*
WC vs HC	0.770, 0.000*	0.685, 0.000*
HC vs WHT	0.779, 0.000*	0.660, 0.000*
WHR vs WHT	0.485, 0.002*	0.638, 0.000*
BMI vs WC	0.205, 0.216	0.566, 0.000*
BMI vs HC	0.295, 0.072	0.562, 0.000*
BMI vs WHT	0.251, 0.128	0.651, 0.000*
AMP vs BMI	-0.629, 0.028	-0.083, 0.743

*=significant; p=probability, r=pearson correlation coefficient, BMI=body mass index, WC=waist circumference, HC= hip circumference, WHR=waist-hip ratio, WHT=waist-height ratio, AM=age at menarche, AMP= age at menopause

Table 2 shows the correlation of demographic with anthropometric indices in females with breast cancer and controls. Positive correlations among height, weight, waist circumference (WC) and waist-hip ratio (WHR); weight and body mass index (BMI) were observed in cases only. Positive correlations were observed among waist circumference (WC) correlated positively with waist-hip ratio (WHR), waist-height ratio (WHT) and hip circumference (HC). Hip circumference correlated positively with waist-height ratio (WHT). Waist-hip ratio (WHR) correlated positively with waist-height ratio (WHT) in both cases and controls. Non-significant negative correlation was observed between age at menopause (AMP) and body mass index (BMI) in both cases and control. Positive correlations were observed among body mass index (BMI) with hip circumference (HC) and waist-height ratio (WHT). Weight however correlated negatively with age at menarche (AM). These correlations were statistically significant ($p<0.05$).

DISCUSSION

Recent studies have shown that the incidence of breast cancer in Nigeria is on the increase in recent years particularly in younger women because of its obscure aetiology (Adesunkanmi *et al.*, 2006; Arinola and Charles-Davies, 2008). The most established risk factors are related to life-time hormone exposure, early menarche, late menopause and recently, exposure to endocrine disrupting substances (Brophy *et al.*, 2012; Fernandez and Russo, 2010; Kortenkamp, 2006). This present study showed that demographic indices (age, age at menarche, age at menopause) are similar in both breast cancer patients (cases) and non- breast cancer patients (controls). This could be because the subjects were age matched.

Statistically significant increases were observed in weight, height, waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), weight-height ratio (WHT) in cases when compared with controls ($p<0.05$). Non-significant increase was observed in body mass index (BMI) in cases when compared with the controls. This signifies that adiposity could be a contributory factor in the aetiology of breast cancer. While WC has been shown as an accurate predictor of visceral adiposity either alone or in combination with BMI, visceral fat remains more associated with increased risk of cancer development (Steffen *et al*, 2009). The systemic effects exerted by visceral adiposity are focus of much research (Donohoe *et al.*, 2011). WHR measurements may be more relevant in determining cardiovascular risk than WC alone, whether this is the case for cancer risk is not yet known (Donohoe *et al.*, 2011). BMI is associated with an increased incidence of many types of cancer including breast cancer (Renehan *et al*., 2008; Donohoe *et al.*, 2011). There is still paucity of information on the involvement of HC and WHT in the aetiology of breast cancer (Donohoe *et al.*, 2011). Positive correlations were observed among WC and WHR, WHT, HC; HC and WHT; WHR and WHT in both cases and controls. However, positive correlations among height, weight, WC and WHR; BMI and weight were observed in cases only. Weight correlated negatively with age at menarche (AM) only in controls. The differences and correlations were statistically significant ($p<0.05$). This is equally a pointer to the fact that adiposity plays a major role in cancer aetiology.

Humans are exposed daily to a variety of compounds, it is thus likely that even if none of these chemicals reach an effective level, the combination or mixture of chemicals may become dangerous. These chemicals enter the food chain and accumulate in animals up to humans (Lubrano *et al.*, 2013). BisphenolA (BPA), a breakdown product of coatings in food and beverage containers, may act as oestrogen receptor agonist. In this present study, there was a significant increase in the serum BPA level in cases when compared with thecontrols. This is in consonance with the outcome of a similar study (Fernandez and Russo, 2010).

In this present study, significant increase was observed in the serum PCB levels in cases when compared with the controls. There is currently paucity of information on females who are non-occupationally exposed, particularly in Nigeria. Some Polychlorinated biphenyls (PCBs) activate Aryl hydrocarbon receptor (AhR) (Lubrano *et al.*, 2013). Although the mechanisms through which AhR regulates energy metabolism are not clearly established, crosstalk with oestrogen receptor may be involved (Lubrano *et al.*, 2013).
Recently, much evidence has emerged indicating that environmental toxicants including BPA and PCBs can affect mitochondrial function and cause pro-oxidative conditions leading to pathological conditions like cancers (Valavinides *et al.*, 2006; Albers *et al.*, 2010; Farahat *et al.*, 2011). This further supports the fact that oxidative stress could be one of the mechanisms of action of these toxicants in the aetiology of breast cancer.

In conclusion, mechanisms involving PCBs and BPA as well as adiposity may be involved in breast cancer aetiology reduction of environmental pollution and dietary modulation may be helpful. Policies aimed at discouraging the practice of packaging of food products or water with materials containing BPA should be put in place by the relevant authority. Adequate maintenance of hazardous waste dumps and city landfills as well as control of illegal or improper dumping of hydraulic fluids/coolants, leaks from electrical transformers and other equipment, burning of medical, industrial or city waste from older consumer goods like televisions may reduce

environmental pollution due to PCBs. These strategies together with consumption of healthy diet and lifestyle may prevent, protect and reduce the high mortality associated with breast cancer in Nigeria.

REFERENCES

Adesunkanmi AR., Lawal OO, Adelusola KA, Durosimi MA (2006). The severity, outcome and challenges of breast cancer in Nigeria. *Breast*.15(3): 399-409

Albers G, Echteld MA, de Vetite, Onwuteaka-Philipsen BD, van der Linden MH, Deliens L (2010). Evaluation of quality of life measures for use in palliative care: A systematic review. *Palliative Med*. 24:17-37

Arinola OG and Charles Davies MA (2008). Micronutrient levels in the plasma of Nigerian females with Breast cancer. *African Journal of Biotech*.7:11.1620-23

Betancourt Angela M, Wang Jun, Sarah Jenkins Sarah, Mobley Jim, Russo Jose, and Lamartiniere Coral A (2012). Altered Carcinogenesis and Proteome in Mammary Glands of Rats after Prepubertal Exposures to the Hormonally Active Chemicals Bisphenol A and Genistein. J. *Nutr*.142: 7 1382S-1388S

Brisken C (2008). Endocrine disruptors and breast cancer.*Chimia*. 62(5).406-9

Brody JG, Moysich KB, Humblet O, Attfield KR, Beehler GP, Rudel RA (2007).Environmental pollutants and breast cancer: epidemiologic studies.*Cancer*.109(12 Suppl)**:**2667-2711

Brophy James T**,** Keith Margaret M**,** Watterson Andrew**,** Park Robert**,**Gilbertson Michael**,** Maticka-Tyndale Eleanor**,** Beck Matthias**,** Abu-Zahra Hakam**,**Schneider Kenneth**,** Reinhartz Abraham**,** DeMatteoRobert and Luginaah Isaac (2012).Breast cancer risk in relation to occupations with exposure to carcinogens and endocrine disruptors: a Canadian case–control study. *Environmental Health*. 11:87. doi:10.1186/1476-069X-11-87

Calafat Antonia M**,** KochHolger M**,** Swan Shanna H**,** Hauser Russ**,**Goldman Lynn R**,** Lanphear Bruce P**,** Longnecker Matthew P**,** Rudel Ruthann A**,** Teitelbaum Susan L**,** WhyattRobinMandWolff Mary S (2013).Misuse of blood serum to assess exposure to bisphenol A and phthalates. *Breast Cancer Research*.15:403. doi:10.1186/bcr3494

Chakraborty R and Bose K (2009). Central adiposity, body mass index and percent body fat among Bengalee Hindu Male slum dwellers of dumdum, West Bengal, India. *The open Obesity Journal*.1:32-37

Donohoe CL, Doyle SL, Reynolds JV (2011). Visceral adiposity, insulin resistance and cancer risk.Diabetology and Metabolic Syndrome. 3(12) doi: 10.1186/1758-5996-3-12

Farahat FM, Ellison CA, Bonner MR, McGarriagle BP, Crane AL, Fenske RA (2011). Biomarkers of chlorpyrifos exposure and effect in Egyptian cotton field workers. *Environ Health Persp*.119:801-806

Fernandez SV, Russo J (2010).Estrogen and xenoestrogens in breast cancer. *ToxicolPathol*. 38(1)**:**110-122

Gray J, Evans N, Taylor B, Rizzo J, Walker M (2009): State of the evidence: the connection between breast cancer and the environment. *Int J Occup Environ Health.*15:43-78

Haighton LA, Hlywka JJ, Doull J, Kroes R (2002). An evaluation of the possible carcinogenicity of BPA to humans. *Regulatory Toxicol and Pharmacol*.35**:**238-54

Halberg N, Wernstedt-Asterholm L, Schere PE (2008). The adipocyte as an endocrine cell.*Endocrinol. Metab. Clin*. North Am.37:753-68

Iyengar M (2005). Polychlorinated biphenyls-a review.Pp 1-24

Kang SW, Rane WS, Kim SJ, Garrison JL,Taunton J, Heqde RS (2006).Substrate-specific translocation attenuation during ER stress defines a pre-emptive quality control pathway. *Cell*.1:27(5) 999-1013

Kortenkamp A (2006). Environmental contaminants and breast cancer: The growing concerns about endocrine disrupting chemicals. A briefing paper for WWF-UK.Pp 1-8

Lubrano L.,Genovesi G, Specchia, P., Constatini D, Stefania, M, Petrangeli E, Lenzi A, Gnessi L (2013). Obesity and metabolic comorbidities; *Oxidative Medicine and Cellular Longetivity*.Article ID 640673 pp 1-9. http;//dx.doi.org/10.1155/2013/640673

Msolly A, Gharbi O., Mahmoudi K, Limem S, Hochlef M, Ben Ahmed S (2011). Association between body mass index and the risk of breast cancer in Tunisian women. *Annals of Saudi Medicine*.31(4):393-397.

Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M (2008). Body mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet.* 371:569-578

Steffen A., Schulze MB, Pischon T, Dietrich T, Molina E, Chirlaque MD, Barricarte A, Amiano P, Quiros JR., Tumino R (2009). Anthropometry and oesophageal cancer risk in the European Prospective investigation into cancer and nutrition. *Cancer Epidemiol Biomarkers Prev*.18: 2079-2089

Valavinides AT, Vlahogianne M, Dassenakis, Scoullos (2006).Molecular biomarkers of oxidative stress in aquatic organism in relation to toxic environmental pollutants.*Ecotoxicol Environ*. 64.178-189.