People's Democratic Republic of Algeria
Higher Education Ministry And Scientific Research
Echahid Hamma Lakhdar El-Oued University
Faculty of Natural Sciences and Life
Department of Cellular and Molecular Biology

Master's thesis

In order to obtain a diploma of an Academic Master
in biological sciences
Specialty: Applied Biochemistry

Theme

Epidemiological study, predictor factors and prognostic significance of blood biomarkers in El-Oued breast cancer patients

Presented by: ATOUSSI Naouel
GUEDIRI Safa

Thesis Approved: 05h June 2018

Examining Committee:

President	**AOUIMEUR Meriem**	**M.A.A.**	**El-Oued University**
Examiner	**LAICHE Ammar Touhami**	**M.A.A.**	**El-Oued University**
Supervisor	**Dr. DEROUICHE Samir**	**M.C.A.**	**El-Oued University**

2017/2018

Acknowledgement

First, I thank "Allah", for giving us strength, patience and courage to complete this work.

We like to express our gratitude to **Dr. DEROUICHE Samir** for agreeing to supervise us, for his dynamism, his help and his precious advice, allowed us to go further in our research.

We thank Ms. **AOUIMEUR Meriem** who has given us the honor to accept the presidency of this thesis. Respectful tribute.

We thank Mr. **LAICHE Ammar Touhami** for having agreed to examine our thesis and the honor for his presence. Permit us to express to him our deep respect.

We also thank the members of the Ben Omar Djailani Hospital, Fajr Association, Faculty of Natural Sciences and Life of Echahid Hamma Lakhdar El-Oued University, CRSTRA, Agronomy department Biskra, Esalam Center.

We do not forget to express our thanks to my friend and member of our teem **KAOUACHI Aicha** thanks for your encouragement, to everyone who contributed to this work.

Abstract

Breast cancer is the most frequent cancers for woman and represents the first cause of cancer death for woman, in this perspective the aim of our work is carried out an epidemiological study, evaluation of some risk factors and biological markers in patients with breast carcinoma in El Oued region.

Our epidemiological study was carried out on 1505 cancer patients in the period (2007-2017) of the El Oued region obtained from Oncology service of the hospital Ben Amor Djilani and El Fajer association for breast cancer-El Oued. So 100 women (50 healthy women and 50 breast cancer patients) were used to study socio-clinical risk factors and 36 control and cancerous woman for biological markers study (biochemical, minerals and oxidative stress) for screening and follow-up breast cancer.

The results of epidemiological study, show that several types of cancer are marked in different region of El Oued state and that breast, intestine and lungs cancer are the most dominant (24.91%, 10.98 % and 8.57%) respectively, age 41-60 years and women sex are most affected by diseases. for breast cancer, 375 cases were recorded, 28.95% patients were aged between 41-50 years, 2% of breast cancer are in men versus 98% are in women. In this study, 48% of the breast cancer patients had a tumor affecting the right breast whereas 52% in the left breast.

Our study reports shows a strong association between socioeconomic behavior (such as passive smoking, Sunshine exposed...) and clinical history (such as Chronic diseases, contraceptive pulls...) with breast cancer but remains the Phone in Bras and Fast Food the most dangerous risk factor, (OR = 31.06, OR =19.05) respectively, for breast cancer. While Spices and breastfeeding more than 8 months are important protective factors against this disease.

Biological study shows a remarkable change of hematological, oxidative stress and some minerals markers and GSH in erythrocyte (AUC=0.93), leukocyte (AUC=0.875) and salivary (AUC=0.732) and serum ORAC (AUC=0.823) more susceptible to disease. Increase of serum lead level in patients (more than 18 times than controls) is an important indicator of the effect of the environment on the disease.

In conclusion, Several socioeconomic, clinical and environmental factors contributed to the dispersion and development of the breast cancer in the region of El-Oued, suggesting that GSH and ORAC testing be used as systematic markers for screening and followed breast cancer treatment.

Key words: Breast cancer, Risk factors, Oxidative stress, Breastfeeding.

الملخص

سرطان الثدي هو أكثر أنواع السرطانات انتشارا عند النساء ويمثل السبب الأول للموت بالنسبة للمرأة، ومن هذا المنطلق، فإن هدف عملنا هو إجراء دراسة احصائية وبائية لهذا المرض وتقييم بعض العوامل الاجتماعية والمرضية لخطر الاصابة به وكذلك تقييم بعض المعايير البيولوجية في الكشف ومتابعة مرضى سرطان الثدي في منطقة الوادي.

أجريت دراستنا الوبائية على 1505 مريض بالسرطان في الفترة الممتدة من (2007-2017) لمنطقة الوادي، تم الحصول عليها من مصلحة الأورام بمستشفى بن عمر جيلاني-الوادي وجمعية الفجر لسرطان الثدي -الوادي، استعملنا أيضا 100 امرأة متطوعة (50 سليمة و 50 مصابة بسرطان الثدي) لدراسة عوامل الخطر الاجتماعية والمرضية ، من جهة اخرى ولتحديد المؤشرات البيولوجية اجريت هذه الدراسة على 36 امرأة متطوعة بين سليمة (كشاهدة) و مصابة بالمرض حيث تم لهن تقدير بعض المعايير (البيوكيميائية، والمعادن، والاجهاد التاكسدي) وذلك لفحص ومتابعة سرطان الثدي.

من خلال الدراسة الوبائية النتائج المتحصل عليها أظهرت أن عدة أنواع من السرطان تم تحديدها في مناطق مختلفة من ولاية الوادي وأن سرطان الثدي والأمعاء والرئتين هم الأكثر انتشاراً بنسب مختلفة (24.91٪، 10.98٪ و8.57٪) على التوالي، كذلك الفئة العمرية من41 الى 60 سنة هي الأكثر عرضة للمرض، من جهة اخرى تم تسجيل 375 حالة لسرطان الثدي في نفس المنطقة والفترة، حيث 28.95 ٪ من المرضى تتراوح أعمارهم بين 41-50 سنة كما تم تسجيل 2٪ من مرضى سرطان الثدي هم من الرجال مقابل 98 ٪ من النساء على ان اغلب المصابين حدد لديهم الورم في الجهة اليمنى من الثدي بنسبة 48 ٪ مقابل 52 ٪ في الجهة اليسرى منه.

الدراسة الاحصائية لعوامل الخطر اظهرت بوضوح وجود ارتباط قوي بين الاصابة بسرطان الثدي والسلوك الاجتماعي-الاقتصادي (مثل التدخين السلبي، التعرض لأشعة الشمس...) وبعض العوامل الكلينيكية للمرض (مثل الأمراض المزمنة، حبوب منع الحمل...)، ويبقى سلوك وضع الهاتف في حمالات الصدر والافراط في تناول وجبات الاكل السريع هما العاملان الأكثر خطورة (OR = 31.06 وOR =19.05) على التوالي بالنسبة للإصابة بسرطان الثدي، في حين أن الدراسة اظهرت ان استعمال التوابل في الاكل والرضاعة الطبيعية لأكثر من 8 أشهر هما عاملا حماية مهمان ضد هذا المرض.

نتائج الدراسة البيولوجية للمصابات بسرطان الثدي اظهرت تغييرا كبيرا في معايير الاجهاد التاكسدي ومكونات الدم، وبعض المعادن بالنسبة للمريضات مقارنة بالشواهد واظهرت ايضا ان مؤشر الاجهاد التأكسدي GSH فى كريات الدم الحمراء (AUC=0.93)، كريات الدم البيضاء (AUC=0.875)، اللعاب (AUC=0.732) وايضا مؤشر ORAC في المصل (AUC=0.823) هم الأكثر تحسسا واهمية من حيث الكشف ومتابعة سرطان الثدي. كما ان النسبة العالية لمستوى الرصاص في مصل المرضى (مرتفعة باكثر من 18 مرة مقارنة بالأشخاص السليمين) هي مؤشر مهم لتأثير البيئة على المرض في منطقة الوادي.

في الختام، ساهمت العديد من السلوكات الاجتماعية والاقتصادية وبعض العوامل الكلينيكية والبيئية في انتشار وتطور سرطان الثدي في منطقة الوادي وعليه و بالموازات مع استعمال الاشعة نقترح استعمال معيار GSH وORAC في الدم كمؤشرات بيولوجية مهمة من حيث الكشف، فحص ومتابعة علاج سرطان الثدي عند النساء.

كلمات مفتاحية: سرطان الثدي، عوامل خطر، اجهاد تأكسدي، الرضاعة الطبيعية.

Abbreviation list

8-OHDG : 8-hydroxydeoxyguanosine.

Akt: Protein kinase B.

AP-1: Activator-protein-1.

AUC: Area Under Curve.

BC: Breast Cancer.

BCS: Breast-conserving surgery.

BM: Basement Membrane.

BSA: Bovine serum albumin.

Ca: Calcium.

CAT: Catalase.

CEA: carcinoembryonic antigen.

Cl:chlorine.

CNS :central nervous system.

CS : Cigarette smoke.

CT: computed tomography.

Cu SO4 : copper sulphate.

Cu-Zn: cuivre-zinc.

DBT: digital breast tomosynthesis.

DNA: deoxyribonucleic acid.

EDTA : Ethylenediaminetetraacetic acid.

DTNB: 5,5′-Dithiobis(2-nitrobenzoic acid).

ETS : environmental tobacco smoke.

FNS: Hematological analysis.

GI: gastrointestinal.

GOT: Glutamic oxaloacetic transaminase.

GPT: Transaminase Pyruvic Glutamic.

GPx: Glutathione Peroxidase.

GRx: Glutathione Reductase.

GSH: reduced glutathione.

GSSG: oxidized glutathione.

GST: Glutathione S-transferases.

H_2O^+: Ionized Water.

H_2O_2: Hydrogen Peroxide.

H: Hydrogen Radical.

HBV: Hepatitis B Virus.

HCT: Hematocrit.

HCV: Hepatitis C Virus.

HDL-cho: High Density Lipoprotein-Cholesterol.

HGB: Hemoglobin.

HIV: Human Immunodeficiency Virus.

HNO_3: Nitric acid.

HO˙: Hydroxyl Radical.

HO2˙: Hydroxyperoxyl Radical.

HPA: Hypothalamic–Pituitary– Adrenal.

IGF-1: Insulinlike Growth Factor 1.

IL-1: Interleukin-1.

IR: Ionizing radiation.

K: Potassium.

LEP: Luminal Epithelial Cells.

LDL-cho: Low Density Lipoprotein-Cholesterol.

LHRH: Luteinizing Hormone- Releasing Hormone.

MAPKs: Mitogen-Activated Protein Kinase.

MDA: Malondialdehyde.

MEP: Myoepithelial Cells.

METC: Mitochondrie Electrone Transport Chain

MRI: Magnetic resonance imaging.

Na: Sodium.

NaCl: Sodium chloride.

NADPH oxidase: nicotinamide adenine dinucleotide phosphate-oxidase.

NFκB: Nuclear Factor-kappa B.

-NH2: Amino groups.

NK: Natural Killer.

O_2: Oxygen.

OD: Optical Density.

OR: Odds ratio.

ORAC: Oxygen radical absorbance capacity.

OS: Oxidative stress.

P53: Protein p53.

PAHs: Polycyclic aromatic hydrocarbons.

Pb: Lead.

PET: Scans Positron Emission Tomography.

PMS: Premenstrual Syndrome.

RBC: Red Blood Bell.

Redox: Reductive-oxidative.

ROS: Reactive Oxygen Species.

SH: Sulfhydryl.

SOD: Superoxide Dismutase.

TBS: Tris Buffer Saline.

TNF: Tumor Necrosis Factor.

Tris: Tishydroxyméthylaminométhan.

UV: Ultraviolet.

UVA: Ultraviolet A.

UVB: Ultraviolet B.

VLDL: Very Low Density Lipoprotein.

WBC: White globule cell.

WHO: World Health Organization.

Zn: Zinc.

Zn-SOD: zinc-Superoxide dismutase.

FIGURES LIST

TABLES LIST

SUMMARY

EXPERIMENTAL PART

I. Materials and methods

Introduction

Introduction

Cancer is a group of diseases involving uncontrolled growth and spread of abnormal cells. Such cells undergo transformations to obtain inexhaustible replication and thus traverse to other organs leading to malignancy (Ashim et al., 2016).

There are over 100 different known cancers that affect humans, The most common cancers are breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer (Shiwani & Saraswathy, 2017). Among females, breast cancer is the most frequently diagnosed form of cancer (Minna et al., 2017) which considered the leading cause of death in many countries (Ana et al., 2014).

Breast cancer is undoubtedly a relevant public health issue (Ana et al., 2014). This type of cancer develops from breast tissue (Pelumi et al., 2017) and can occur at any age (CCF, 2015). According to WHO estimates in 2012, there were 14.1 million new cases and 8.2 million deaths worldwide. The most commonly diagnosed cancers were lung (1.82 million), breast (1.67 million). The most common causes of cancer death were lung cancer (19.51%), liver cancer (9.08%) and breast cancer (6.96%). In Algeria, more than 8100 new cases of breast cancer are recorded each year, the mortality is about 2840 cases per year.

Several risk factors are introduced as causative factors of developing breast cancer, there is sufficient evidence to suggest that genetic susceptibility (Nkondjok & Ghadirian, 2005) 5-10 % of all cases of cancer (Cheeseman et al., 2012), exposure to environmental pollution (such as pesticide and heavy metals) and lifestyle play an important role in the etiology of this disease; including diet, lower age of menarche, late age of first pregnancy, breastfeeding duration, menopause, contraceptive use. Some diet and parts of diet may increase cancer risk or help prevent cancer (Teresa et al., 2015). Many researchers have reported that zinc inhibits the development of cancer and that low serum zinc is associated with several forms of cancer (Eric et al., 1982). Other risk factors which add to the burden of breast cancer are sun exposure, tobacco, physical activity etc (Preetha et al, 2008).

The most important cause suggested to be involved in induction and progression of breast cancer is the oxidative stress as a consequence of impaired balance between pro-oxidants and antioxidants (Gurer-Orhan et al., 2017).

Reductive-oxidative (redox) balance is an essential component of cancer cell homeostasis implicated in cell proliferation, progression, and drug resistance (Wang et al., 2008). Oxidative stress resulting from an imbalance of reactive oxygen species (ROS) and their counteracting antioxidants is mutagenic and thereby promotes cancer progression

(Tomasz et al., 2017). However, excess oxidative stress can inhibit cancer cell growth and lead to cell death, highlighting the importance of cancer cells generation of sufficient reducing potential to maintain an optimal redox balance (Panieri & Santoro, 2016). To counteract excess ROS, cancer cells often up regulate endogenous antioxidant defenses (Ishimoto et al., 2011).

One of ways to identify the breast cancer is self-examination of breast that patient can detect the palpable mass; thus, this method can reduce probability of death by 50% (Zareh et al., 2018). There are several imaging techniques that are available to evaluate the breast. The most widely used and studied modality for breast cancer screening is mammography. Ultrasonography is typically used as an adjunct to mammography for further evaluation of suspicious areas and has been used as a supplement to mammography for women with dense breasts. Magnetic resonance imaging (MRI) is currently used for screening high risk patients in conjunction with mammography (Kimberly et al., 2017).

There are currently no blood-borne biomarkers recommended for breast cancer diagnosis or screening. Although candidate markers such as carcino-embryonic antigen (CEA) and the soluble form of MUC1 protein (CA15-3, CA27.29) (Anna et al., 2017) is the only internationally accepted indicator for treatment monitoring in advanced stages of the disease (Bayo et al., 2017).

Earlier detection and improved treatment options have resulted in high survival rates for early-stage cancer (Siegel et al., 2016). Oncology-directed treatment for breast cancer is individualized to diagnostic, prognostic, and individual factors, and most often is multidimensional, including surgery, radiotherapy, chemotherapy, and hormone therapy (Daniel et al., 2017).

In light of these data, the aim of our work is based on the realization of two following complementary aspects:

The first part is an epidemiological study and the risk factors associated with breast cancer; several types of factors are studied in this context: socioeconomic, environmental and clinical factors.

The second part is an biological study concerns the determination of the variation and specificity of some biochemical, hematological, mineral and oxidative stress markers in the prediction and diagnosis following up of breast cancer in women El-Oued population.

I. Cancer

I.1. Definition

Cancer is an abnormal growth of cells caused by multiple changes in gene expression leading to dysregulated balance of cell proliferation and cell death and ultimately evolving into population of cells that can invade tissues and metastasize to sites, causing significant morbidity and, if untreated, death of the host (Raymond 2007). Cancer is a group of diseases in which there is abnormal cell growth which have the potential to invade other parts of the body. Cancers cells can form a tumor which invades the local tissue (Shiwani & Saraswathy 2017).

I.2. Risk factors

Cancer is caused by both internal factors (such as inherited mutations, hormones, and immune conditions) and environmental/acquired factors (such as tobacco, diet, radiation, and infectious organisms) (Preetha et al., 2008).

I.2.1. Tobacco

Smoking was identified in 1964 as the primary cause of lung cancer in the US Surgeon General's Advisory Commission Report, and ever since, efforts have been ongoing to reduce tobacco use (Preetha et al., 2008).

Tobacco use increases the risk of developing at least 14 types of cancer. Tobacco contains at least 50 carcinogens. For example, one tobacco metabolite, benzopyrenediol epoxide, has a direct etiologic association with lung cancer (Denissenko et al., 1996).

I.2.2. Alcohol

How alcohol contributes to carcinogenesis is not fully understood but ethanol may play a role. Study findings suggest that ethanol is not a carcinogen but is a cocarcinogen (Posch et al., 2004). Specifically, when ethanol is metabolized, acetaldehyde and free radicals are generated; free radicals are believed to be predominantly responsible for alcohol-associated carcinogenesis through their binding to DNA and proteins (Szabo et al., 2007) which can also contribute to tumorigenesis (Aggarwal et al., 2004).

I.2.3. Diet

The extent to which diet contributes to cancer deaths varies a great deal, according to the type of cancer (Willett, 2000). Most carcinogens that are ingested, such as nitrates,

nitrosamines, pesticides, and dioxins, come from food or food additives or from cooking. Heavy consumption of red meat is a risk factor for several cancers, (Bingham et al., 2002; Hogg, 2007) Furthermore, bisphenol from plastic food containers can migrate into food and may increase the risk of breast (Andrew, 2012) and prostate (Ho et al., 2006) cancers. Ingestion of arsenic may increase the risk of bladder, kidney, liver, and lung cancers (Smith et al., 2002).

I.2.4. Obesity

Studies have shown that the common denominators between obesity and cancer include neurochemicals; hormones such as insulin like growth factor 1 (IGF-1), insulin, leptin; sex steroids; adiposity; insulin resistance; and inflammation. Involvement of signaling pathways such as the IGF/insulin/Akt signaling pathway (Hursting et al., 2007). For instance, hyperglycemia, has been shown to activate NF-κB, which could link obesity with cancer (Nareika et al., 2008). Also known to activate NF-κB are several cytokines produced by adipocytes, such as leptin, tumor necrosis factor (TNF), and interleukin-1 (IL-1) (Tang et al., 2007).

I.2.5. Infectious Agents

Worldwide, an estimated 17.8%of neoplasms are associated with infections; Viruses account for most infection (Pisani et al., 1990; Parkin 2006) caused cancers. HIV, HBV, and HCV are associated with risks for cancer (Song et al., 1999), However, other microorganisms, including selected parasites such as *Opisthorchis viverrini* or *Schistosoma haematobium* and bacteria such as *Helicobacter pylori*, may also be involved, acting as cofactors and/or carcinogens (Belpomme et al., 2007).

I.2.6. Radiation

Radiation exposure from various sources including medical treatment and nuclear explosion (Rupen et al., 2014), radioactive substances and ultraviolet (UV), pulsed electromagnetic fields. Increases the risk of cancer. Low-frequency electromagnetic fields can cause clastogenic DNA damage. The sources of electromagnetic field exposure are high-voltage power lines, transformers, electric train engines, and more generally, all types of electrical equipments (Belpomme et al., 2007).

In addition, a recent meta-analysis of all available epidemiologic data showed that daily prolonged use of mobile phones for 10 years or more showed a consistent pattern of an increased risk of brain tumors (Andrew, 2012).

I.2.7. Environmental Pollution

Environmental pollution has been linked to various cancers. It includes outdoor air pollution by carbon particles associated with polycyclic aromatic hydrocarbons (PAHs); indoor air pollution by environmental tobacco smoke, formaldehyde, and volatile organic compounds such as benzene; food pollution by food additives and by carcinogenic contaminants such as nitrates, pesticides, dioxins, and other organochlorines; carcinogenic metals and metalloids; pharmaceutical medicines; and cosmetics (Srogi et al., 2007).

Long-term exposure to chlorinated drinking water has been associated with increased risk of cancer. Nitrates, in drinking water, can transform to mutagenic N-nitroso compounds, which increase the risk of lymphoma, leukemia, colorectal cancer, and bladder cancer (Weyer et al., 2001).

I.3. Types

Tumors can be benign or malignant (NCIS, 2007):

Benign tumors

- Are usually not harmful
- Rarely invade the tissues around them
- Don't spread to other parts of the body
- Can be removed and usually don't grow back

Malignant tumors

- May be a threat to life
- Can invade nearby organs and tissues (such as the chest wall)
- Can spread to other parts of the body
- Often can be removed but sometimes grow back

I.4. Treatment and Prevention

I.4.1. Treatment

I.4.1.1. Surgery and use of modern technology

Ancient surgeons knew that cancer would usually come back after it was removed by surgery. Progress in ultrasound (sonography), computed tomography (CT scans), magnetic resonance imaging (MRI scans) and positron emission tomography (PET scans) have replaced most exploratory operations (Akulapalli, 2009).

Using miniature video cameras and endoscopy, surgeons can remove colon, esophagus and bladder tumors through tubes (Akulapalli, 2009). Cryosurgical techniques are less invasive and have lower morbidity. It is a method for eradicating cancer cells without removing them to prolong life (Yiu et al, 2007). Lasers also can be used to cut the tumor tissue of cervix, larynx, liver, rectum, skin and other organs. The use of lasers in tumor surgery has several advantages: remote application, precise cutting, hemostasis, low cicatrization, reduced postoperative pain (Neukam & Stelzle, 2010).

I.4.1.2. Chemotherapy

Radiotherapy is used before, during, or after surgery and is frequently combined with chemotherapy, either as concurrent or adjuvant treatment (David & Mary, 2015). It has been reported that nitrogen mustards is effective at killing cancer cells due to their DNA-damaging properties (Kahlin et al., 2013). Chemotherapy drugs have historically been tested, dosed, and incorporated alone or combined into treatment of many types of cancers (Sonia et al., 2012). Now new approaches are being studied to reduce the side effects of chemotherapy including use of, (a) new combinations of drugs, (b) liposomal and monoclonal antibody therapy to target specifically cancer cells, (c) chemoprotective agents to reduce chemotherapy side effects, (d) hematopoietic stem cell transplantation and (e) agents that overcome multidrug resistance (Akulapalli, 2009).

I.4.1.3. Hormonal therapy

New classes of drugs are being used to treat prostate and breast cancers. How hormones influence growth of cancer has guided progress in developing as well as reducing the risk of breast and prostate cancers such as aromatase inhibitors, LHRH (Luteinizing Hormone-Releasing Hormone) (ACS, 2014). Therefore, antiestrogens have been suggested as chemopreventive agents to inhibit the development and progression of prostate cancer (Maarten, 2005).

I.4.1.4. Radiation therapy

In 1896, Roentgen discovered "X-ray" and after 3 years later radiation was used for cancer diagnosis and in treatment. Researchers discovered that radiation could cause cancer as well as cure it (Akulapalli, 2009).

I.4.1.5. Adjuvant therapy

Adjuvant chemotherapy is the use after radiotherapy or surgery in curing patients with advanced cancer. Adjuvant therapy was used in colon and testis cancers (Manuel et al., 2011).

I.4.2. Prevention

Cancer prevention comprises primary and secondary prevention. Primary prevention includes the removal of environmental carcinogens (such as tobacco use) and conditions that favor the development of cancer (such as obesity) (ODOVICO, 2016). Chemoprevention is a specific form of primary cancer prevention that encompasses the utilization of agents that oppose carcinogenesis or cancer growth (ACS, 2017). Secondary prevention focuses on the early diagnosis of cancer when malignancy may still be curable or managed with less toxic therapy through the screening of asymptomatic individuals at risk (Basu et al., 2017).

II. Breast cancer

II.1. Anatomy of the breast

The breast is a symbolic organ of femininity, sexuality and motherhood. It is in continuous development from the embryonic stage, through puberty, menstrual cycles and pregnancy, to atrophy through menopause (Zaki et al., 2013).

The breast is an even and globular organ. It occupies the entero-superior part of the thorax (front and upper thorax) (Moinfar, 2007).

This organ is centered by a structure called the nipple which is a projection in which the lactifers open. The nipple is surrounded by a pigmented area is the areola. The breast is traversed by blood vessels and lymphatic vessels. Anatomically, the breast is divided into four quadrants: supero-external, superior-medial, infero-external and infero-internal (CCS, 2018). The biological function of the breast is to produce milk to feed a newborn (WHO, 2009).

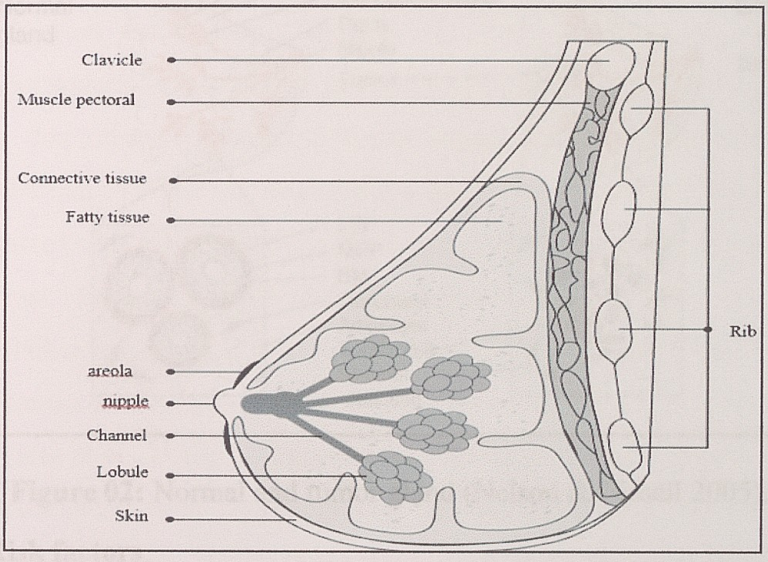


Figure 01: Sagittal section of the breast (CCS, 2015).

II.2. Histology of the mammary gland

The breast is composed of adipose tissue and a mammary gland (located deep on the pectoralis major muscle) (Moinfar, 2007).

The mammary gland is an exocrine gland. Like most compound glands, it is composed of a ductal system, lobes (15 to 20), which are themselves subdivided into lobules (Kierszenbaum, 2006).

The latter comprises a branched system of intra-lobular and inter-lobular excretory ducts that extend into the fibro-adipose tissue of the breast. The galactophore ducts are bordered by a double cellular base: internal consisting of cylindrical or cubic cell, external constituted by a discontinuous layer of myoepithelial cells (which are between the luminal cells and the basement membrane). These channels are surrounded by loose connective tissue containing blood vessels and lymphatics (Kierszenbaum, 2006).

II.3. Breast cancer

Breast cancer is the type of cancer that develops from breast tissue; it is mostly common in women and it is one of the most studied diseases, largely because of its high mortality. However, it occurs in males also (Pelumi et al., 2017). It is a tumor that affects the mammary gland (HAS, 2011).

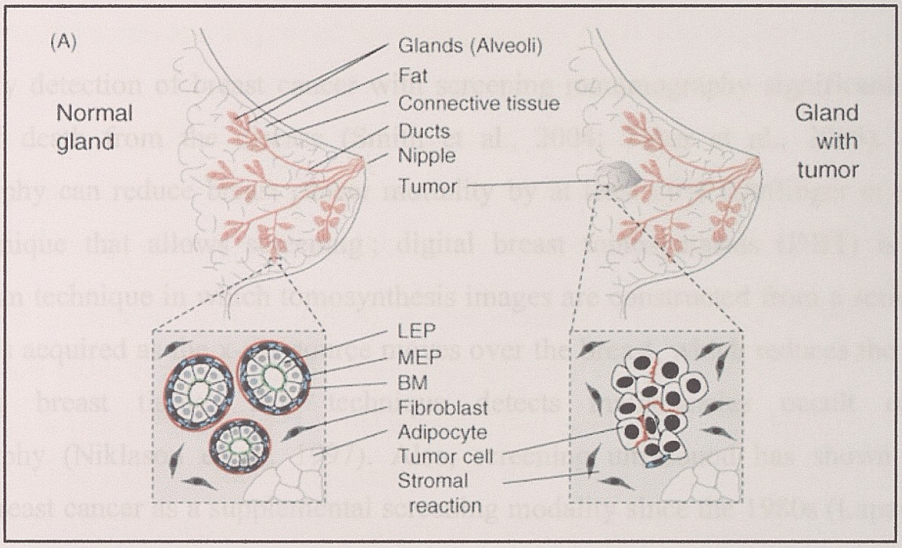


Figure 02: Normal and tumor gland (Nelson & Bissell 2005).

II.3.1. Risk factors

It is very difficult to determine the cause of breast cancer. Studies scientists have shown that some person-specific characteristics or behaviors were more often observed in women who had breast cancer than in other women (DONNA, 2007).

Increasing age is a major risk factor. Risk increases with inherited genetic mutations, such as in the BRCA1 and BRCA2 genes, and a personal or family history of breast cancer (Howell et al., 2014). Other risk factors include never having children, greater height, benign breast disease, early menarche, late menopause (after age 54). ionizing-radiation Exposure, postmenopausal obesity, physical inactivity, and alcohol intake (Hankinson et al., 2008 ;

Petrakis et al., 2006). Breastfeeding, moderate/vigorous physical activity, and maintaining a healthy body weight decrease breast cancer risk (Rachael et al., 2017; Howell et al., 2014). High breast tissue density (Rachael et al., 2017) and high circulating levels of estrogens (Key et al., 2003; WCRF, 2010) have also been associated with higher breast cancer incidence. Abdominal adiposity, as measured by waist circumference and waist-to-height ratio, are also considered risk factors (Canchola et al., 2012; Bernstein et al., 2003).

II.3.2. Breast Cancer Screening

Breast cancer care consists of a multidisciplinary approach of surgery, radiation, and systemic therapy including chemotherapy (Light et al., 2017).

One of ways to identify the breast cancer is self-examination of breast that patient can detect the palpable mass; thus, this method can reduce probability of death by 50% (Zareh et al., 2018).

Early detection of breast cancer with screening mammography significantly reduces the risk of death from the disease (Smith et al., 2004; Tabar et al., 2015). Screening mammography can reduce breast cancer mortality by at least 20% (Oeffinger et al., 2015). Other technique that allows screening ; digital breast tomosynthesis (DBT) is a digital mammogram technique in which tomosynthesis images are constructed from a series of low-dose images acquired as the x-ray source moves over the breast, which reduces the impact of overlapping breast tissue. This technique detects malignancies occult on digital mammography (Niklason et al., 1997). Also, screening ultrasound has shown utility in detecting breast cancer as a supplemental screening modality since the 1980s (Lapayowker & Revesz 1980; Sickles et al., 1983). Compared with screening mammography alone, screening ultrasound in combination with mammography can increase cancer detection (Berg et al., 2008; Weigert & Steenbergen, 2012). In addition, screening breast MR Imaging; Breast MR imaging with gadolinium is highly sensitive ($\geq$ 90%) for the detection of breast cancer (Kuhl et al., 2010; Sardanelli et al., 2011). Screening MR imaging was more sensitive (90%–93%) than clinical breast examination (18%), mammography (33%-50%), ultrasonography (37%-52%), or mammography combined with ultrasonography (48%-63%) (Riedl et al., 2015). MRI has been recommended for women with a >20% lifetime risk of breast cancer (Chetlen, et al., 2015).

II.3.3. Symptoms

Several symptoms are known in people with breast cancer including primary and distant recurrent (Balazs 2015):

There is many recurrent local region such as; Lump in the breast/chest wall/axilla, Dimpling of the skin, Nipple retraction, Clear or bloody nipple discharge (spontaneous), Redness, scaling, thickening of nipple-areolar complex, Rash on breast, unresponsive to antibiotics. Also there is other types of symptoms are related to distant recurrence like New-onset localized bone pain lasting longer than 2 weeks (long bones, ribs, spine), Persistent chest pain, with or without cough, Persistent abdominal pain, Unintended weight loss, Persistent headache, Personality changes, New-onset seizures and Loss of consciousness.

II.3.4. Treatment

Earlier detection and improved treatment options have resulted in high survival rates for early-stage cancer, increasing the number of women living with a history of treatment (Siegel et al., 2016). Oncology-directed treatment for breast cancer is individualized to diagnostic, prognostic, and individual factors, and most often is multidimensional, including surgery, radiotherapy, chemotherapy, and hormone therapy (Daniel et al., 2017).

Breast-conserving surgery (BCS) is the standard treatment for early-stage breast cancer patients (Rao et al., 2018). At one time, the only choice was whether to remove all (mastectomy) or part (lumpectomy) of the breast (Tulin et al., 2017). Chemotherapy intents to eliminate potential existing micrometastases, thus decreasing recurrence rates and mortality (Collaborative, 2005). Chemotherapy consists of using drugs (by injection into a vein most often) that act on all cancer cells, even those that have not been detected by imaging tests (CCI, 2007). Breast-conserving surgery followed by radiation therapy is a widely accepted standard approach that allows for organ preservation in most early stage breast cancers (Serguei et al., 2017). Radiotherapy uses X-rays to destroy cancer cells in the breast or in certain lymph nodes (CCI, 2007).

III. Oxidative stress

III.1. Definition

Stress oxidative defined as "A disturbance in the pro-oxidant and anti-oxidant balance in favor of the former, leading to potential damage" (Alfonso et al., 2003). Under normal physiological condition, oxidants are removed through antioxidant defense mechanism. If incompletely cleared by antioxidants, oxidants will cause accumulation of ROS. In efficiency and insufficiency of antioxidant defense system are concerned in some pathological conditions induced by ROS (Sarawoot & Phanit, 2015).

ROS has been implicated in a wide array of diseases such as neurodegenerative disorders, autoimmune diseases, complex life style diseases and cancer (Shilpa & Rima, 2017).

III.2. Free radicals

III.2.1. Free radical definition

A free radical is a chemical species (atom or molecule) containing one or more unpaired electrons in outer orbit and is capable of independent existence. The odd number of electron(s) of a free radical makes it unstable, short lived and highly reactive. Because of their high reactivity, they can abstract electrons from other compounds to attain stability. Thus the attacked molecule loses its electron and becomes a free radical itself, beginning a chain reaction cascade which finally damages the living cell (Alugoju et al., 2015; Sergio & Alberto, 2013). However, the term "free radicals" is frequently replaced by "reactive oxygen species" (ROS), which is a more general term and includes both free radical and non-radical species (Volodymyr, 2014) as defined in Table 01. Reactive oxygen species can be formed due to effect of both endogenous and exogenous factors. Endogenous ROS formation is a consequence of metabolic and other biochemical reactions (Rinne & Nik, 2004).

Table 01: Reactive oxygen species.

Radicals		**Non- radicas**	
Superoxide	O_2•	Hydrogen peroxide	H_2O_2
Hydroperoxyl	H_2O_2 •	Peroxynitrite[a]	ONOO-
Hydroxyl	OH•	Peroxynirous acid[a]	ONOOH
Peroxyl	RO_2•	Nitrosoperoxycarbonate[a]	$ONOOCO_2^-$

Alkoxyl	RO•	Hypochlorus acid	HOCl
Carbonate	CO_3 •-	Hypobromus acid	HOBr
Carbon dioxide	CO_2 •-	Ozone	O3
Singlet oxygen	O_2 [1]		

[a] Also called reactive nitrogen species

III.2.2. Free radical source

III.2.2.1. Endogen Source of Oxidants

Mitochondria are recognized as the major site for ROS production (Lenaz, 2012) and both complexes I and III have been established to be the specific sites for mitochondrial ROS generation (Rakesh et al., 2014). Besides mitochondria, many enzymes are also capable of producing ROS. These include, but not limited to, NADPH oxidase, xanthine oxidase (Agarwal et al, 2011), ketoglutarate dehydrogenase complex, d-amino acid oxidases, and dihydrolipoamide dehydrogenase (Zhiyou & Liang-Jun, 2013). Other potential endogenous sources, microsomes, inflammatory, neutrophils, eosinophils, and macrophages (Fatma et al., 2013).

III.2.2.2. Exogenous Source of Oxidants

a. Ultraviolet light

UV-light is composed of two major components; UVA (320-400 nm) and UVB (290-320 nm). UVA inflicts more noticeable oxidative stress through ROS formation than UVB because the exposition to UVB is much lower than to UVA due to the UVB absorption by ozone layer. UV-light also causes depletion of endogenous antioxidant systems (Xiao, 2012).

Damage induced by UV radiation can be caused by direct or indirect mechanism. When living system is exposed to UV radiation, UV-light energetic photons are absorbed by a cellular molecule (chromophore, photosensitizer). Direct absorption of UV photons by cellular chromophores (for example DNA bases) can lead to photo-induced reactions. Indirect way includes the photosensitization step (Alena et al., 2006).

b. Ionising radiation

In living cells, ionizing radiation (IR) causes an increase in the ROS levels in two different ways.

IR initially causes ionization and excitation of H_2O, leading to the formation of various H_2O radiolysis products such as hydrated electron (eaq-), ionized water (H_2O^+),

hydroxyperoxyl radical ($HO_2^{\cdot}$), hydroxyl radical ($HO^{\cdot}$), hydrogen radical ($H^{\cdot}$) and hydrogen peroxide (H_2O_2) in a very short period of time . Except H_2O_2, these formed radicals are unstable and disappear within less than (Tohru et al., 2012).

c. Xenobiotics

Xenobiotic is term used for all chemical compounds foreign to human body containing drugs, food additives or environmental pollutant (Dibyajyoti, 2014). ROS generation induced by xenobiotics include direct metabolization of xenobiotics to primary radical intermediates and activation of endogenous sources of ROS such as METC (James et al., 2011).

Other drugs (analgesic drug paracetamol or antineoplastic drug cisplatin) may cause depletion in cell antioxidant systems and raise the harmful effect of generated ROS (Carla & Stefano, 2013).

d. Cigarette Smoke

Cigarette smoke contains many oxidants and free radicals and organic compounds, such as superoxide and nitric oxide (Rashmi et al., 2017).

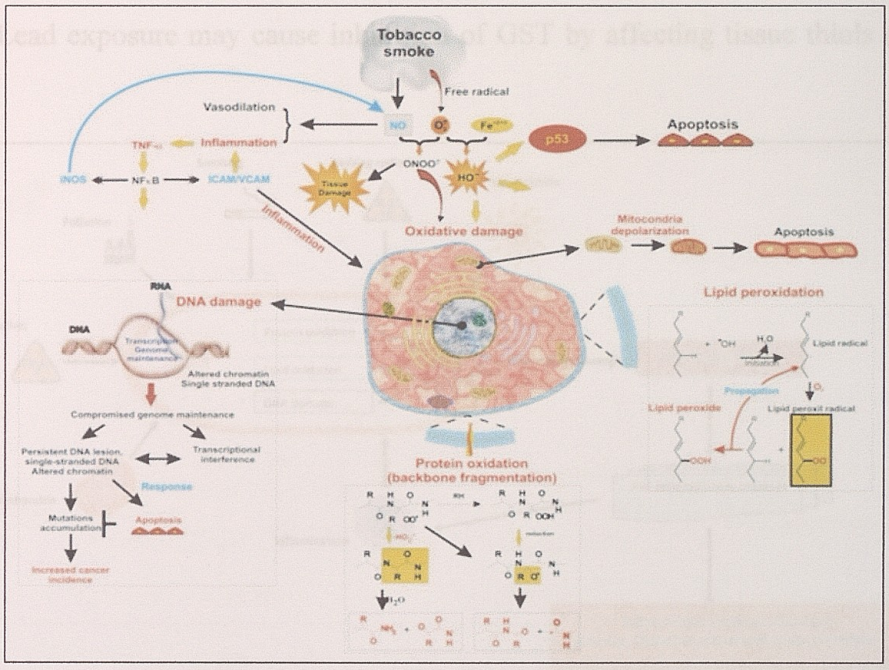


Figure 03: Tobacco smoke and oxidative stress (Peter et al., 2010).

e. Heavy Metal Ions

Metals like iron, copper, cadmium, mercury, nickel, lead and arsenic possess the ability to generate reactive radicals, resulting in cellular damage like depletion of enzyme activities, damage to lipid bilayer and DNA (Flora et al., 2008).

One of the most important mechanisms of metal mediated free radical generation is via a Fenton-type reaction.Superoxide ion and hydrogen peroxide can interact withtransition metals, such as iron and copper, via the metal catalyzed Haber–Weiss/Fenton reaction to form OH radicals (Esra et al., 2012).

$$Metal^{+3} + O_2 \longrightarrow Metal^{+2} + O_2 \quad \text{Haber-Weiss}$$

$$Metal^{+2} + H_2O_2 \longrightarrow Metal^{+3} + OH^- + OH^{\cdot} \quad \text{Fenton reaction}$$

Besides the Fenton-type and Haber–Weiss-type mechanisms, certain metal ions can react directly with cellular molecules to generate free radicals, such as thiol radicals, or induce cell signaling pathways. These radicals may also react with other thiol molecules to generate O^-_2 which is converted to H_2O_2, which causes additional oxygen radical generation (Leonard et al., 2004).

Lead increases lipid peroxidation (Monterio et al., 1990). Significant decreases in the activity of tissue SOD and brain GPx have been reported after lead exposure. Replacement of zinc, which serves as a cofactor for many enzymes by lead, leads to inactivation of such enzymes. Lead exposure may cause inhibition of GST by affecting tissue thiols (Esra et al., 2012).

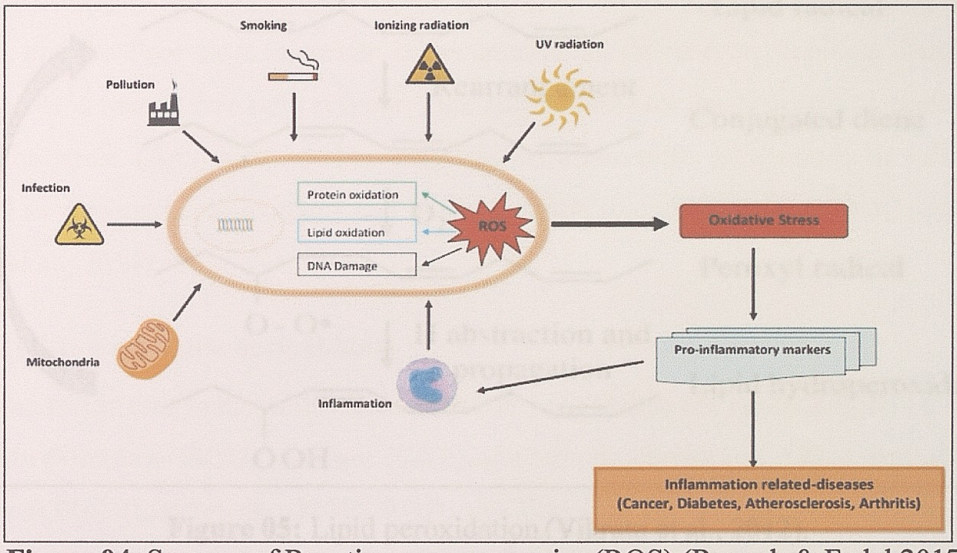


Figure 04: Sources of Reactive oxygen species (ROS) (Ranneh & Fadel 2017).

III.2.3. Free radical damage

III.2.3.1. Effects of Oxidative Stress on lipids

Lipids that contain phosphate groups (i.e., phospholipids) are essential components of the membranes that surround the cells and cell structures. Unsaturated phospholipids of cell

membrane are sensitive to oxidation. The most common fatty acid in cells is linoleic acid (Ulla & Peeter, 2015).

The hydroxyl radical extracts a hydrogen atom from a methylene carbon of a polyunsaturated fatty acid forming a carbon-centered lipid radical. The lipid radical can interact with molecular oxygen to give rise to a peroxyl radical. The peroxyl radical is converted to a lipid hydroperoxide by extracting a hydrogen atom from a methylene carbon to form a new lipid radical, thus, initiating a propagating chain of lipid peroxidation (Thorburne & Juurlink, 1996).

Products of lipid peroxidation, such as MDA and unsaturated aldehydes, are capable of inactivating many cellular by forming protein cross-linkages (Esra et al., 2012). and are mutagenic products of lipid oxidation and can further react with DNA (Klaunig et al, 2010) This peroxidation chain is generally ended when the lipid radicals interact with vitamin E forming a lipid alcohol and a tocopherol radical (Ulla & Peeter, 2015).

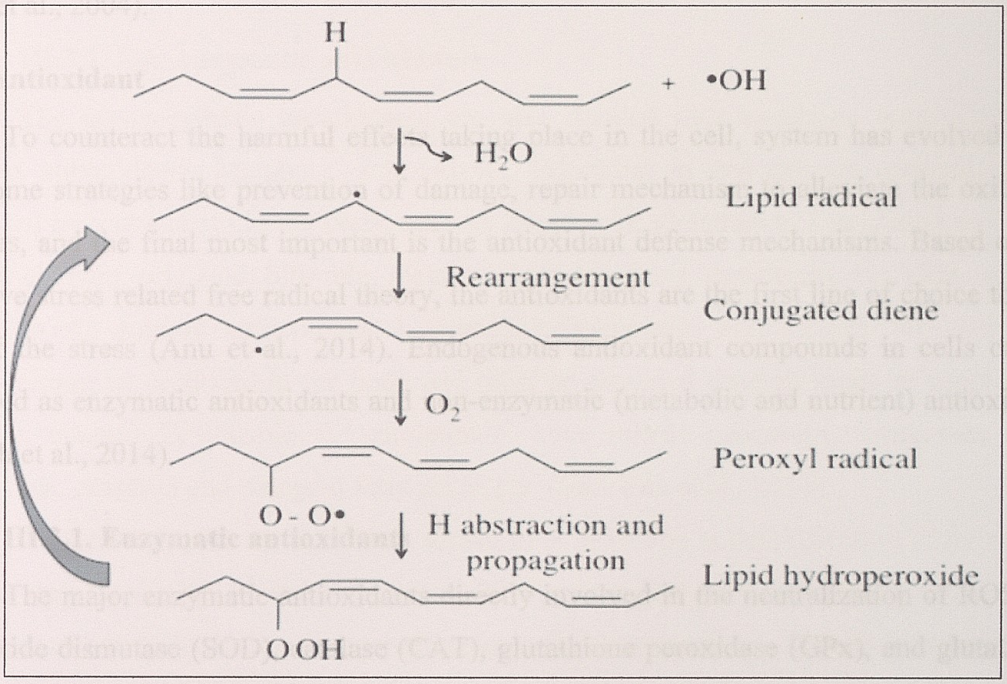


Figure 05: Lipid peroxidation.(Vikram et al., 2012).

III.2.3.2. Effects of Oxidative Stress on Proteins

The most sensitive proteins to radical attacks are especially those which comprise a sulfhydryl (SH) group (Favier, 2003).

ROS can cause fragmentation of the peptide chain, alteration of electrical charge of proteins, cross-linking of proteins and oxidation of specific amino acids and therefore lead to increased susceptibility to proteolysis by degradation by specific proteases (Ozougwu, 2016).

Cysteine and methionine residues in proteins are most susceptible to oxidative modifications (Shihong et al., 1995). Oxidation of sulfhydryl groups or methionine residues of proteins cause conformational changes, protein unfolding, and degradation (Esra et al., 2012).

III.2.3.3. Effects of Oxidative Stress on DNA

Although DNA is the memory of the entire bio-chemical composition of living beings, it is a molecule that is very susceptible to attack by oxygen radicals (Favier, 2003).

ROS can create various types of DNA damage; modification of all bases, deletions, frame shifts, strand breaks, DNA-protein cross-links, and deoxyribosebackbone and chromosomal rearrangements. $^{\bullet}OH$ and $ONOO^{-}$ in particular can react with all components of DNA and form several new compounds (Ulla & Peeter, 2015).

One of these will generate 8-hydroxydeoxyguanosine (8-OHdG), which has been implicated in carcinogenesis and is considered a reliable marker for oxidative DNA damage (Saroj et al., 2004).

III.3. Antioxidant

To counteract the harmful effects taking place in the cell, system has evolved itself with some strategies like prevention of damage, repair mechanism to alleviate the oxidative damages, and the final most important is the antioxidant defense mechanisms. Based on the oxidative stress related free radical theory, the antioxidants are the first line of choice to take care of the stress (Anu et al., 2014). Endogenous antioxidant compounds in cells can be classified as enzymatic antioxidants and non-enzymatic (metabolic and nutrient) antioxidants (Rakesh et al., 2014).

III.3.1. Enzymatic antioxidants

The major enzymatic antioxidants directly involved in the neutralization of ROS are: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRx) (Lien et al., 2008). SOD, the first line of defence against free radicals, catalyzes the dismutation of superoxide anion radical ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed (H_2O_2) is transformed into water and oxygen (O_2) by catalase (CAT) or glutathione peroxidase (GPx). The selenoprotein GPx enzyme removes H_2O_2 by it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotei enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or non-lipid hydroperoxides while oxidizing glutathione (GSH) (Paramasivam et al., 2012).

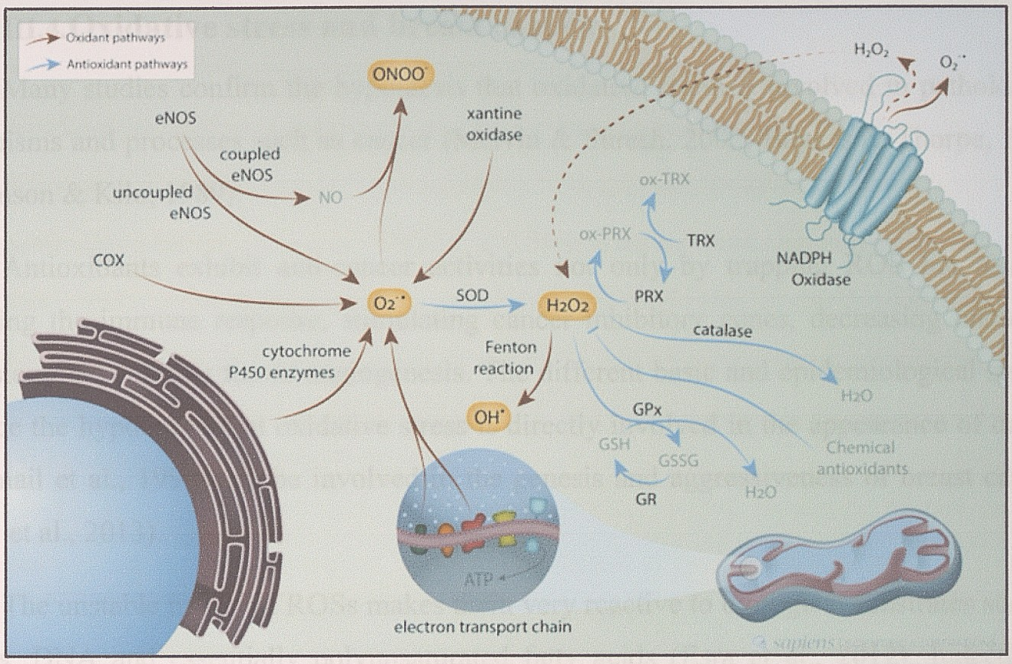


Figure 06: Enzymatic antioxidant defense mechanisms (Fabio et al., 2016).

III.3.2. Non-enzymatic antioxidants

The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants, belonging to endogenous antioxidants, are produced by metabolism in the body, such as lipoid acid, glutathione, L-ariginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc (Rakesh et al., 2014). While nutrient antioxidants, belonging to exogenous antioxidants, are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, etc (Jiao-Kun et al., 2016).

Nutrient antioxidants have been shown to be involved in detoxification of the reactive oxygen species (ROS) (Rakesh & Neeta, 2013). Also, play an important role in helping endogenous antioxidants for the neutralization of oxidative stress. The nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies and cancer (Lien et al., 2008).

III.4.Oxidative stress and breast cancer

Many studies confirm the hypothesis that oxidative stress is involved in pathological mechanisms and processes such as cancer (Melvin & Suresh, 2002; Baynes & Thorpe, 1999; Williamson & Kilo, 1989).

Antioxidants exhibit anti-cancer activities not only by trapping ROS but also by increasing the immune response, stimulating cancer inhibitory genes, decreasing oncogene expression, or inhibiting tumor angiogenesis. The different basic and epidemiological studies reinforce the hypothesis that oxidative stress is directly involved in the appearance of cancer (Pincemail et al., 1999). To be involved in the genesis and aggressiveness of breast cancer. (Tahari et al., 2013).

The unstable nature of ROSs makes them very reactive to biological substrates such as proteins, DNA and essentially polyunsaturated fatty acids (Esra et al., 2012). Indeed, the attack of the proteins by the ROS can modify their catalytic functions, as it is the case of the activation of "Nuclear Factor-kappa B" (NFκB) and "Activator-protein-1" (AP-1) that are involved in proliferation and cell survival and inactivation of phosphatases that negatively regulate these two processes (Hung-Chi et al., 2015). Oxidation of DNA can give rise to point mutations in tumor suppressor genes or in proto-oncogenes (Randy, 2004).

In the case of breast cancer, cancer cells have a high rate of mutations in mitochondrial DNA and a genetic instability of antioxidant enzymes inducing an increase in the invasiveness of these cells. The accumulation of these mutations is mainly associated with a deficiency of some DNA repair proteins, including p53 and BRCA1 or 2 (Karihtala et al., 2006).

IV. Metal and breast cancer

IV.1. Lead

IV.1.1. Definition

Lead is a chemical element that exists in nature (Derouiche & Djouadi, 2017). It is heavy metal with a bluish-gray color (WHO, 2010). It does not really have a smell or a taste. It is a highly toxic substance, which has no physiological role in the body (Almeras et al., 2013). Exposure to lead produces a variety of adverse health effects in populations through its impact on different organs and systems (Bruce, 1993).

IV.1.2. Sources of exposure to lead

Lead is found at low levels in Earth's crust (IARC, 2006). However, the widespread occurrence of lead in the environment is largely the result of human activity. such as mining, smelting, refining and informal recycling of lead, use of leaded petrol (gasoline), production of lead-acid batteries and paints, jewellery making, soldering, ceramics and leaded glass waste, manufacture in informal and cottage (home-based) industries, electronic (WHO, 2010) and use in water pipes and solder. Water distribution systems, and lead-soldered food and soft drink cans (Loghman-Adham, 1997).

IV.1.3. Metabolism

IV.1.3.1. Absorption

a. Ingestion

Accidental ingestion of lead compounds may occur. Lead may be ingested on contaminated food, drinks and cigarettes, or lead particles trapped in the upper respiratory tract may be swallowed. 5-15% of ingested inorganic lead is absorbed from the gastro-intestinal tract; the rest passes through the body unabsorbed, and is eliminated in the faces. (IPCS, 1994) this Absorption rate can increase to as much as 45% under fasting conditions (Putnam, 1986).

Absorption higher in children than in adults and is lower in the presence of food (WHO, 2011). Decreased calcium zinc, selenium or phosphate may also increase of lead absorption gastrointestinal absorption of lead occurs by acid solubilization and it seems that lead transport across the digestive mucosa is similar to that of calcium (Gilman, 1990).

b. Inhalation

Following inhalation, deposition of lead within the respiratory tract and its subsequent absorption are determined by ventilatory rate, particle size and solubility (PHE, 2017). Deposition in adults has been estimated at between 30-50% of inhaled lead. Particles smaller

than 1 μm have been shown to have greater deposition and absorption rates than larger particles as they are able to reach the lower respiratory tract where absorption appears to be complete (FAO/WHO,2011). Larger particles (i.e. over 5 μm) are deposited in the trachea and bronchi. From there they may be cleared from the respiratory tract by mucociliary transport and subsequently ingested, leading to potential absorption from the gastrointestinal (GI) tract (EFSA, 2010).

c. Dermal exposure

Skin absorption is not considered a significant mode of exposure among the general population. Organic lead is more likely to be absorbed through the skin than inorganic lead, and mostly occurs among people who work closely with it (Heda & Danielle, 2014).

IV.1.3.2. Distribution

Lead will primarily partition to blood, soft tissues and bone. The half-life of lead in blood is approximately 35 days (Michael et al., 1976). However, bones act as a reservoir for lead, with a biological half-life of approximately 20–30 years (lyn, 2006).

Under normal conditions, most lead (> 98%) is bound to cellular proteins within red blood cells. Thus, this lead is not available for crossover to other tissues (CDW, 2017). The remaining lead can be found as complexes with low molecular weight sulphydryl compounds (e.g., cysteine and homocysteine) within serum and as protein-bound lead (e.g., to albumin and γ-globulins) within plasma. Although present in only small quantities, the lead in plasma is the most biologically available for uptake by other tissues (ATSDR, 2007). Small amounts of lead have been found to permeate several tissues, including liver, kidney, skeletal muscle, pancreas, ovary, spleen, prostate, adrenal gland, brain, fat, testis and heart, with higher levels observed in bone, hair and nails. Of the soft tissues, aorta, liver and kidney retained the most lead, as shown in human cadavers (CDW, 2017).

IV.1.3.3 Excretion

Lead is primarily excreted through urine and feces; other minor pathways include hair, nails and breast milk. The proportions of lead excreted through each of these pathways will vary according to the exposure route (CDW, 2017).

IV.1.4. Toxicity

Lead interacts with bio systems causing dysfunctionat the level Molecular and cellular processes as well as in the cell signaling process. Lead contamination in mammals causes toxicity and numerous biochemical, physiological and behavioral dysfunctions (Bukola et al.,

2015). Hepatic, kidney (Ponce-Canchihuaman et al., 2010), haematological, such as anemia or neurological behavior (WHO, 2010). Also, previous studies have shown several health problems as cardiovascular disease and high blood pressure appears due to exposure to lead at low doses (Derouiche et al., 2017a).

IV.1.5. Lead and oxidative stress

The main mechanism of heavy metals toxicity has been attributed to oxidative stress (Flora et al., 2008). Is a state that involves the generation of free radicals beyond the permitted limits. Free radicals in lipid peroxidation, cell membrane disruption, protein oxidation, and oxidation of nucleic acids and RNA leading to cancer (Gagan et al., 2012).

IV.1.6. Lead and cancer

Lead is a genotoxic agent causes genotoxicity by oxidative stress in exposed cells, tissues and organs. Beside this, lead is also reported to cause impairment in DNA synthesis process and cause chromosomal aberrations (Aftab et al., 2015) and destabilization of DNA , abnormal base pairing, formation of micronuclei, chromosome aberration, and sister chromatid exchanges (Clement et al., 2015).

Many of lead genotoxic effects in mammal cells are mediated by ROS and/or the lipids soluble byproducts of oxidative stress such as MDA (Bertrand et al., 2011). In a cellular system, it has been demonstrated that singlet oxygen is the major species participating in the induction of DNA strand breakage and 8-hydroxydeoxyguanosine adduct induced by lead (Jia-Ling et al., 1999). In addition, OH^* is considered to be the ultimate reactive oxygen species which interacts with DNA and promotes genetic damage. The OH radical attacks DNA on the sugar residue and induces DNA fragmentations, base loss and strand breaks with a terminal sugar residue fragment (Bertrand et al., 2011).

IV.2.Zinc

IV.2.1. Definition

Zinc found in group IIb, in the periodic table of elements with the two toxic metals cadmium and mercury (Laura et al., 2010). Which is essential trace element that exists in all cells (Derouiche et al., 2017b) and plays essential roles in metabolisms from human and animals (Ahmadi-Vincu, 2005).

IV.2.2. Physiological role of zinc

Zinc is an essential trace element not only for humans, but for all organisms. it is a component of more than 300 metallo-enzyme (Derouiche et al., 2017b). By participating in their structure or in their catalytic and regulatory actions (Maria et al., 2002). Zinc play indispensable role for human health. Optimal nucleic acid and protein metabolism, as well as cell growth, division, and function, require sufficient availability of zinc (Laura et al., 2010), development and proliferation, since DNA replication and transcription require the activity of zinc-dependent polymerase and transcriptase enzymes (Prasad, 1996).

IV.2.3. Zinc and oxidative stress

Zinc plays a role as an antioxidant indirect in ensuring the stabilization of the formed Cu-Zn showings (jemaia et al., 2007). Zinc inhibits the production of reactive oxygen species (ROS) by the transition metals (Claeyssen et al., 2009). It protects thiol groups (SH) of proteins agains oxidation induced by iron. By preventing the formation of intra-molecular disulfide bridges (Patricia, 2012). Zinc also inhibits the enzyme NADPH oxidase which catalyzes the production of O_2^- from O_2 (Ananda, 2007).

IV.2.4. Pathology of zinc deficiency

Zinc toxicity is not a major health problem. In the other hand, due to its essentiality, a lack of this trace element leads to far more severe and widespread problems (Laura et al., 2010). The symptoms of Zinc deficiency is characterized by growth retardation, And impaired immune function. In more severe cases, zinc deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions. Weight loss and impaired appetite, delayed healing of wounds, taste abnormalities, and altered cognition can also occur have been associated with zinc deficiency (Josko & Natasa, 2011).

Materials and Methods

I. Materials and methods

I.1. Patients and reagents

I.1.1. Study period

Our study was conducted over a period of 7 months (from the beginning of September 2017 to the end of March 2018) at Oncology service and Medical Analysis Laboratory of the hospital of Ben Amor Djilani. Faculty of Science of Nature and Life at the University of Echahid Hamma Lakhdar El-Oued.

I.1.2. Epidemiological study and questionnaire

For Epidemiological study The cancer reports of 1505 patients (For registered cancer patients between 2007 and 2017) from El Fajr Society for Cancer Patients and oncology service of Ben Amor Djilani Hospital in El Oued state, Algeria were collected and information such as the patient's name, age, sex, type of cancer and treatment provided was noted. The data was compiled to get the percentage distribution and graphs were drawn for the individual data.

For the questionnaire and the statistical study our work is based on 100 voluntary individual divided into 50 healthy women reserved as a control and 50 female cancer patients with mean age 44.15±0.91 years their origin covers the whole El Oued region.

I.1.3. Biological study

For biological study, This work is carried out on 36 volunteers women of age between 25 -80 years, were divided into two groups; a group of 20 healthy control women with mean age 42.82 ± 1.20 year, the other group of 16 women has breast cancer hospital Ben Amor Djilani with mean age 45.48 ± 1.37 year.

Inclusion criterion

- ✓ Voluntary women live in the El Oued region.
- ✓ Control women in good health, does not have any pathology.
- ✓ Women suffering from breast cancer (Diagnosis by mammography Annex 36-37)

Exclusion criterion:

- x Women have other types of cancer.
- x Women suffering from other chronic pathology.

I.1.4. Reagents

Ethylenediaminetetraaceticacid (EDTA), Hydrogen Peroxyde (H_2O_2), Hydrochloricacid (HCl), Thiobarbituric acid (TBA), Methanol, Tris, Salicylic acid,

Trichloroacetic acid (TCA), coppersulphate($CuSO_4$), Ascorbic acid, Nitric acid (HNO_3), Sodium chloride (NaCl), Lead acetat ($Pb(NO_3)_2$), Coomassie Blue, Phosphate-buffered (KH_2PO_4,K_2HPO_4), Butylated hydroxytoluene (BHT), Saline, Zinc nitrat ($Zn(O_2CCH_3)_2$, Phosphoric acid (H_3PO_4), DTNB (5,5′-Dithiobis(2-nitrobenzoic acid)).

I.2. Methods

I.2.1. Collection of data

All voluntary individual answered a questionnaire (Annex38) including social and clinical data that can give us different factor associated with the pathology.

I.2.1.1. Sample collection

Performed blood sampling for both groups is done morning fasting. It is performed on the vein of the bend of the elbow. After the blood sampling, the blood is collected in two tubes:

- Dry tubes are centrifuged at 3000 rpm for 10 minutes then recover the serum to achieve dosage:
  - Biochemistry parameter: Glucose, urea, creatinine, totale bilirubin, direct bilirubin, cholesterol, triglyceride, HDL-cho, LDL-cho, VLDL,
  - Enzymatic parameter: Amylase, alkaline phosphatase, GPT, GOT.
  - Metal analysis: zinc, lead, iron, Ca, Na, Cl, K in Serum, zinc in hair and lead in soil.
  - Blood Oxidative stress parameters: MDA, GSH, Catalase, and antioxidant "ORAC".
- The anticoagulant tube (EDTA) is mixed well and then assays the hematological parameters.

I.2.2. Determination of Zinc concentration

I.2.2.1. In serum samples

In the serum samples, zinc was determined after 10-fold dilution. In this case, the zinc standards were prepared from a 1 mg/mL zinc nitrate standard solution, All tubes were soaked in HNO_3 (10% v/v).

I.2.2.2. In Hair samples

Cut a random piece from a patient and healthy woman's hair. Put in Erlenmeyer flask, 0.1g of hair, 4ml with nitric acid. Warm up to the hotplate at 75 ℃ for 25 min, Then allow to cool. After cooling 1 ml of oxygenated water is added. Other times Heat the hotplate to 40° C until all the bubbles drop and then increase the temperature to 85° C. wash 3 times with 1.5 ml distilled water. Finally do the dosage of zinc (Onuwa et al., 2012).

I.2.3. Determination of lead level

I.2.3.1. In the serum samples

In the serum samples, lead was determined after 10-fold dilution. In this case, the lead standards were prepared from a 1 mg/mL lead acetate standard solution, All tubes were soaked in HNO_3 (10% v/v).

I.2.3.2. In soil samples

Soil samples had been taken in different regions of the El-Oued (Algeria) and some neighboring areas. Each sample was placed in a plastic bag and numbered.

Soil samples are dried in an oven at 40°C for at least 16 hours. It is then shredded on a 2 mm sieve. Then mineralized; is carried out on about exactly 0.5 g of this powder with 6 ml of hydrochloric acid and 2 ml of nitric acid. This step is done at 95°C for 75 minutes on a heating block. Obtained liquid was used for these lead assays. Lead standards were prepared from a 1 mg/mL lead acetate standard solution. All tubes were soaked in HNO_3 (Nicolas, 2007).

I.2.4. Biochemical parameter assay

Serum glucose ,urea, creatinine, bilirubin, Calcium, iron and lipid parameters levels were determined by Autoanalysis (BIOLIS24j) use commercial kit from Spinreact, Spain (ref:glucose-20121, urea-20141, creatinine-20151 ,Total biliribin-20103, Direct biliribin-20102, calcium: 20051, iron-20061, cholesterol-20111, triglyceride-20131, HDL -20113) and enzyme marker were also measured using commercial kits (Spinreat, ref: alkaline phosphates-20015, a amylase -20031,GOT-20042,GPT-20046)

I.2.5. Method of Hematological analysis

Hematological analysis (FNS) is performed by the hematology Autoanalyzer (Sysmex).

I.2.6. Method of electrolytes analysis

Determination of the ionogram parameter (sodium, potassium and chlorine) by Automatic electrolyte analyzer (Easylute).

I.2.7. Determination of saliva Protein concentration

I.2.7.1. Saliva collection

The saliva collected in dry tube was made fasting morning. It was centrifuged at 3000 rpm for 10 minutes. Recovered supernatant for protein and oxidative stress assay.

I.2.7.2. Principle

Protein concentration was measured according to the method of Bradford M.M., 1976 that uses Comassie blue as reagent. The latter reacts with the amino groups ($-NH_2$) of the proteins to form a blue complex (The appearance of the blue color reflects the degree of ionization of the medium and the intensity corresponds to the concentration of the proteins).

I.2.7.3. Operating mode

- Take 0.02 ml of saliva.
- Add 1 ml of Coomassie Blue.
- Shake and let stand for 5 min for color stabilization.
- Read the optical density at 595 nm, against the control.
- The obtained optical density is reported on a calibration curve previously drawn. (Annex35)

The concentration of the proteins is determined by comparison with a standard range of bovine serum albumin (1 mg / ml) previously carried out under the same conditions.

I.2.8. Method of estimating oxidative stress parameters

I.2.8.1. Preparation of erythrocyte homogenate

Blood EDTA tubes contents are centrifuged at 2000 rpm for 10 min and removed the plasma. The cap of EDTA tube was lysis with 50 ml of TBS buffer (EDTA 2.92M; tris 1.21M; pH=7) and incubated 30 min in Freezer. After incubation centrifuged at 2500 rpm for 10 min, and the obtained supernatant (erythrocyte homogenate) was used for the determination of antioxidant activity (Miller et al., 1988).

I.2.8.2. Leukocyte separation

After separation of erythrocyte, the rest of EDTA tube contents centrifuge at 2000 rpm for 10 min and removed the plasma. Wash pellet with lysis buffer and shake incubate in Freezer for 30 min. After incubation centrifuged at 2500 rpm for 10 min. followed this step by

washing with lysis buffer until the Leukocyte pairing and then recovered to make the dosage of stress tests (Miller et al., 1988).

I.2.8.3. Malondialdehyde (MDA) Assay

MDA was measured according to the method described by (YAGI, 1976). Thiobarbituric acid 0.67% (w/v) was added to a liquots of the sample previously precipitated with 10% trichloroacetic acid (w/v). Then the mixture was centrifuged, and the supernatant was heated (100°C) for 15 min in a boiling water bath. Then cool in a cold water bath for 30 minutes leaving the tubes open to allow evacuation of the gases formed during the reaction and the absorbance was measured at 532 nm using a spectrophotometer.

The concentration of TBARS was determined using the molecular extinction coefficient of MDA ($a = 1.53\ 10^5\ M^{-1}\ .Cm^{-1}$).

I.2.8.4. Reduced glutathione (GSH) Assay

The determination of the reduced glutathione concentration by measuring the optical density results from the formation of 2-nitro-5-mercocapturic acid from the reduction of dithio-bis-2-nitrobenzoic acid, which is called reagent of Ellman with SH groupings exist in GSH briefly, 800 µL of erythrocyte homogenate are added to 200 µL of salicylic acid (0.25%) and centrifuge at 1000 rpm for 5 minutes. 500 ml of supernatant are then mixed with 1000 µl of tris buffer (tris 0.4 mol, 0.02 mol NaCl pH = 8.9) and 25 µL of DTNB (0.01 $mol.L^{-1}$). After 5 minutes of incubation, the absorbance is read at 412 nm (WEAK & CORY, 1988).

$$GSH(nM/mg\ of Hb) = \frac{OD \times 1 \times 1.525}{13133 \times 0.8 \times 0.5 \times \text{mg of Hb}}$$

13133: Absorption constant of SH groups at 412 nm.

OD: the absorbance reader by the spectrophotometer.

1.525 ml: total volume of blend.

0.5 ml: volume of solution float.

1: volume of protein mixture.

0.8ml: volume of homogeneous solution without protein exists in 1ml.

GSH: concentration of glutathione.

I.2.8.5. Measuring of total antioxidant capacities (ORAC)

a. Principle

The total antioxidant power of the serum, that is to say its capacity to absorb oxygen free radicals (ORAC: Oxygen Radical Absorbance Capacity) is estimated by the ability of red blood cells to resist free radical induced hemolysis in vitro in the presence of plasma according to the method of. This method is based on the time-dependent monitoring of red blood cell hemolysis induced by a free radical generator (Blache & Prost, 1992).

b. Treatment of RBC

❖ Centrifuge donor blood at 2000 rpm for 10 min and remove plasma.

❖ Wash gently 1 volume of the pellet with 2 volumes of physiological saline (without lysing the RBCs), then centrifuge again at 2000rpm for 5 min.

c. Operating mode

➢ **Control tube**

- To 1 ml of RBC add: 20 µl of $CuSO_4$ (2 mM), 20 µl of H_2O_2 (30%) and 2 ml of physiological saline, then stir gently.
- Incubate for 5 min at room temperature, centrifuge for 5 min at 2000 rpm.
- Read the OD at 450 nm from the supernatant, put it back in the tube, and stir gently.
- Repeat this operation every 10 minutes for 2 hours.

➢ **Standard tube**

- To 1 ml of RBC are added: 20 µl of $CuSO_4$ (2 mM), 20 µl of H_2O_2 (30%) and 2 ml of physiological saline, and 20 µl of vitamin C (400 µM) and then gently stir.
- Incubate for 5 min at room temperature, centrifuge for 5 min at 2000 rpm.
- Read the OD at 450 nm from the supernatant and put it back in the tube and stir gently.
- Repeat this operation every 10 minutes for 2 hours.

➢ **Test tube**

- To 1 ml of RBC are added: 20 µl of $CuSO_4$ (2 mM), 20 µl of H_2O_2 (30%) and 2 ml of physiological saline, and 20 µl of serum and then gently stir.
- Incubate for 5 min at room temperature, centrifuge for 5 min at 2000 rpm.
- Read the OD at 450 nm from the supernatant and put it back in the tube and stir gently.
- Repeat this operation every 10 min for 1 hour (t0, t10, t20, t30, t40, t50, t60, and average the latter:

- **ΣDO = Σ(t0, t10,t20, t30, t40, t50, t60)/7**
- To calculate the total antioxidant power using two methods.

Calculated

$$ORAC(UI) = \frac{\sum(\text{ODcontrol} - \text{ODsample})\Delta t}{\sum(\text{ODcontrol} - \text{ODstandard})\Delta t}$$

I.2.8.6. Determination of catalase activity

The catalase activity consists in measuring the catalase-induced H_2O_2 disappearance contained in the sample by measuring the absorbance of H_2O_2 at 560 nm using a UV / visible spectrophotometer. Briefly in test tubes, mix 1 ml of phosphate buffer (0.1 mHg, 0.1M, pH7.2), 0.975 ml of freshly prepared H_2O_2 (0.091M) and 0.025 ml of the enzyme source (homogenate). Absorption read at 560 nm every minute for 2 minutes (Aebi, 1984).

$$CAT(UI/g) = \frac{\left(\left(\frac{2.3033}{T}\right) \times \left(\frac{\log A1}{\log A2}\right)\right)}{g\ of\ protein}$$

A1: absorbance at the first minute

A2: absorbance at the second minute

T: time interval in minutes

I.2.9. Statistical analysis

Data were reported as mean ± SEM. Statistical analysis is performed by the **SPSS V20.0** software results comparisons were carried out by using Student t test to compare means among the groups, Correlation analysis was carried out using Pearson Correlation test and regression analysis was used for other analysis and statistical data. Differences were considered statically significant at $p<0.05$.

Results

II. Results

II.1. Epidemiological study of Cancer in El-Oued region

II.1.1. Study of all type of cancer

In this study, all type of cancer was estimated about 1505 patients in El Oued region.

II.1.1.1. Distribution of patients by type of cancer

Our results (figure 07) show that the region of El Oued characterized by the existence of different types of cancer with different percentage and that breast cancers is the most common type (24.91%). Following intestin cancer (10.89%), lung (8.57%) and liver (8.17%) respectively.

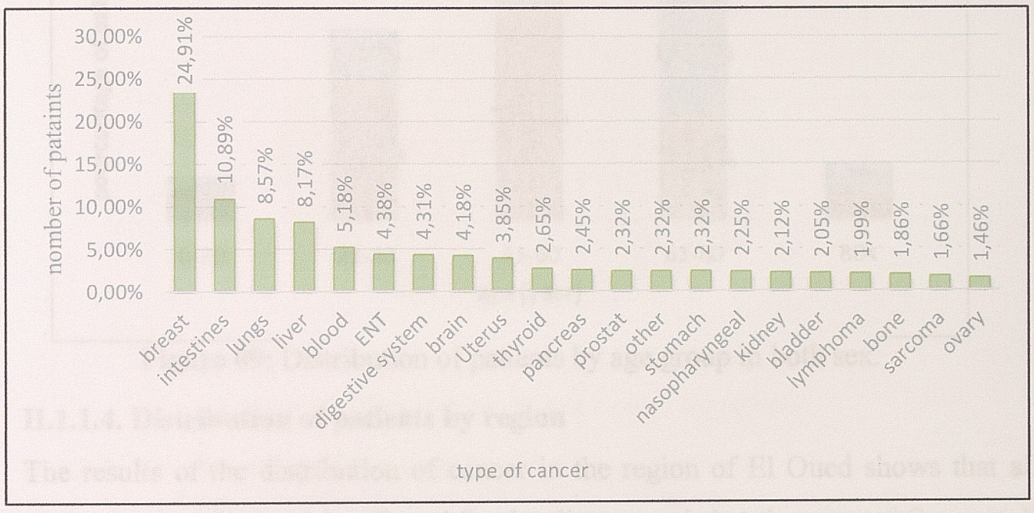


Figure 07: Distribution of patients by type of cancer.

II.1.1.2. Distribution of cancer by sex

Figure 08 illustrates the number of cases for different types of cancers by sex; shows that more than partial of cancer number found in women (58.74%) than men (41.26%).

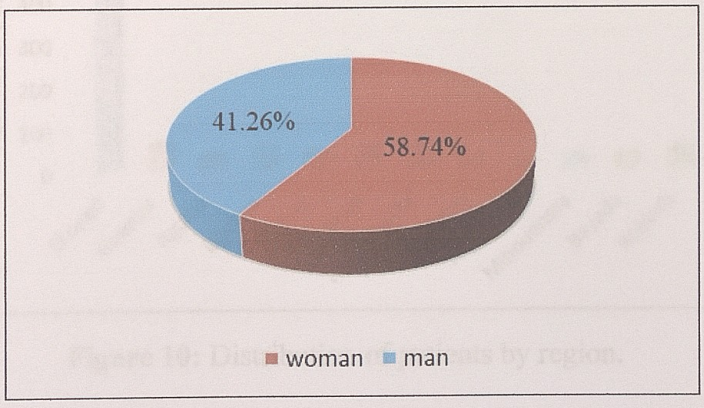


Figure 08: Distribution of cancer by sex.

II.1.1.3. Distribution of patients by age group in both sex

The results presented in Figure 09 clearly shows that all age groups are affected by the different types of cancer in men and women. That age group 41-60, 61-80 and 21-40 year consist of large numbers of patients (36.12%, 27.84% and 23.26%) respectively in all population study, in the same population it has been found that the age group 41-60 is more dominant for women but the 61-80 age group is the most dominant for men.

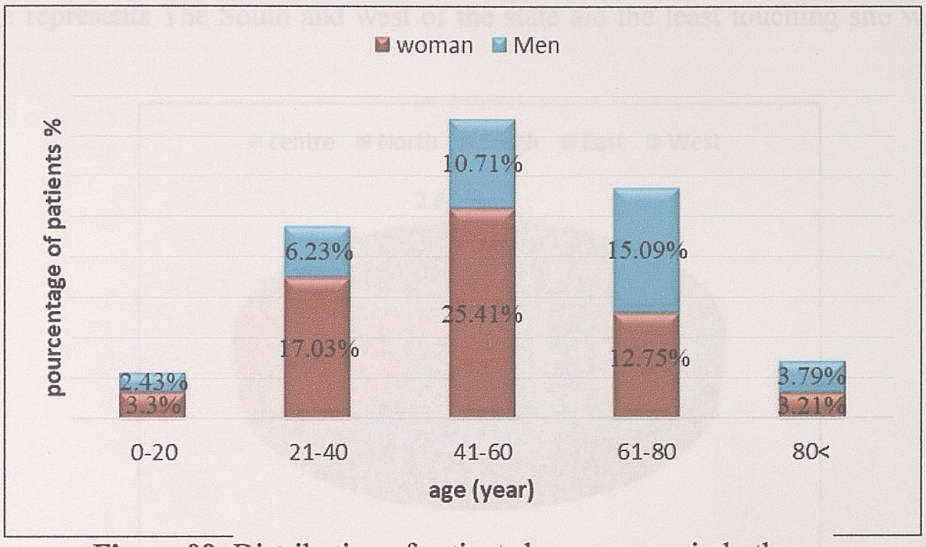


Figure 09: Distribution of patients by age group in both sex.

II.1.1.4. Distribution of patients by region

The results of the distribution of cancer in the region of El Oued shows that all the region of the state of El-Oued is affected by the disease and that the area of Guemar, Hassi khalifa, Dbila and Djammaa are the most dominant concerning the numbers of cancer patients.

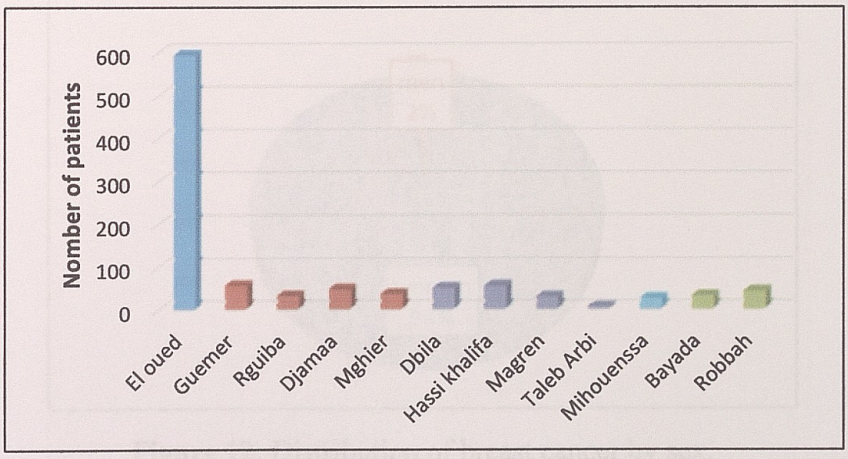


Figure 10: Distribution of patients by region.

II.1.2. Study of breast cancer

In this study, the number of patients was about 375 patients registered in El Oued.

II.1.2.1. Distribution of breast cancer by region

According to graphical presentation in figure 11, the center of the state is the site the most affected by breast cancer than the others sites; with a higher frequency (57.93%), followed by North and East which accounts 17.42% and 14.50% respectively. The lowest percentage represents The South and west of the state are the least touching site with breast cancer.

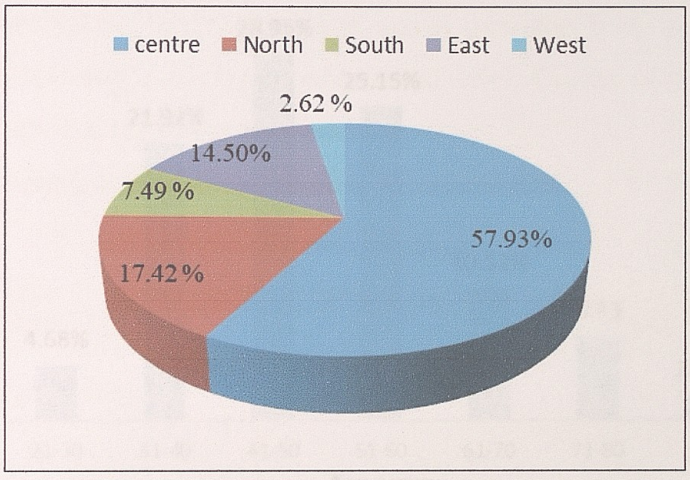


Figure 11: Distribution breast cancer by region.

II.1.2.2. Distribution of breast cancer by sex

As shown in the figure 12, the development of breast cancer in the region of El Oued is more frequent in women with approximately 98% of cases where men is represented 2% of the cases.

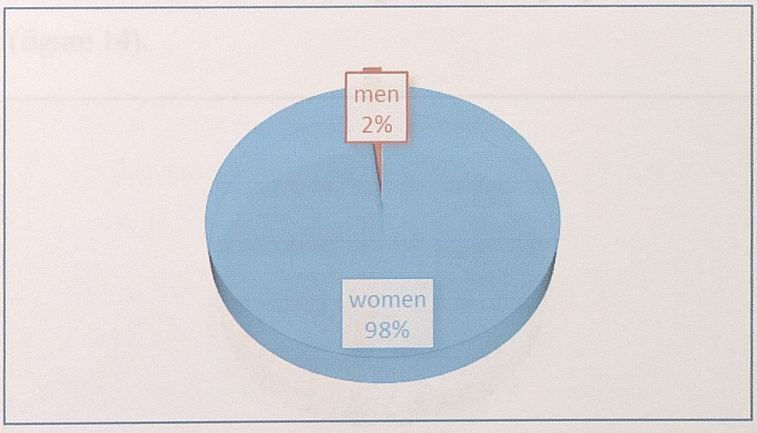


Figure 12: Distribution of breast cancer by sex.

II.1.2.3. Distribution of breast cancer patients by age

In this study, it was found that there were no breast cancer patients between the age groups 0-20 years of age. 4.68 % of the people between 21-30 years, 21.92 %of the people between ages 31-40 years, 28.95 % of people between 41-50 years, 25.15 %of people between 51-60 years, 10.52 % of people between 61-70 years, 6.73 % between 71-80 years and 2.05 % of people more than 80 years were found to be affected by breast cancer in El Oued population (figure 13).

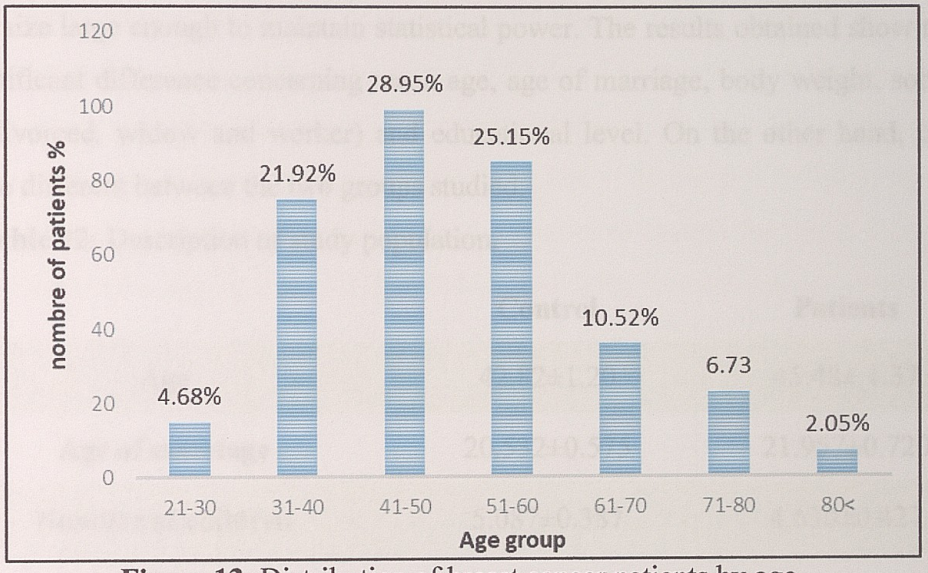


Figure 13: Distribution of breast cancer patients by age.

II.1.2.4. Breast Cancer incidence in right or left breast

In this study, it was observed that 48% of the breast cancer patients had a tumor affecting the right breast whereas the remaining 52% of the people were affected due to tumor in the left breast (figure 14).

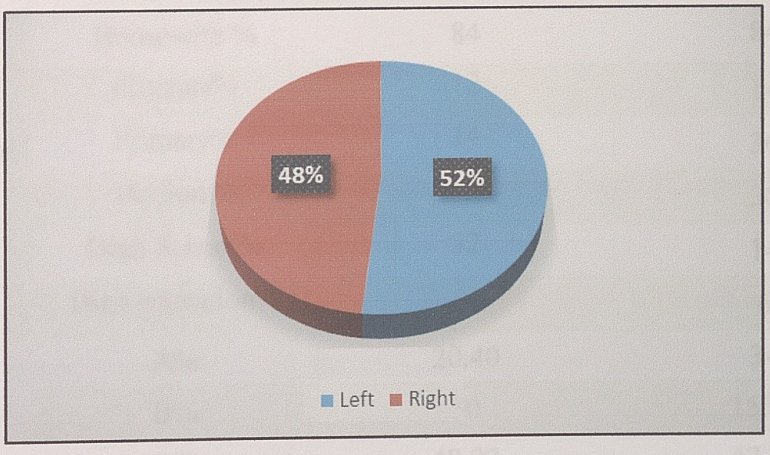


Figure 14: Breast cancer incidence in right or left breast

II.1.3. Study of predictors factors of breast cancer

II.1.3.1. Descriptive of study population

Characteristics of the study population are shown in Table 02. Women volunteers for this study are recruited in hospitals. The recruitment of breast cancer cases is based on confirmed breast cancer diagnosis by mammography (Annex36,37) and by the specialist doctors of the hospital Benamor Djilani - wilaya of El Oued. After a women's agreement to participate in this study, the selected population reaches 50 control and 50 cancerous women, a sample size large enough to maintain statistical power. The results obtained show that there is no significant difference concerning mean age, age of marriage, body weight, social cases (single, divorced, widow and worker) and educational level. On the other hand, the blood groups are different between the two groups studied.

Table 02: Description of study population.

		Control	Patients
Age		42.82±1.20	45.48± 1.37
Age of marriage		20.532±0.575	21.957±0.720
Number of children		5.087±0.387	4.630±0.427
Weight		73.40±1.67	72.48±2.07
Social case	Married%	88	86
	Single%	6	10
	Divorced%	4	2
	Widow%	2	2
Job	Worker%	16	16
	Housewife %	84	84
Educational level :	Illiterate%	12	14
	Primary%	14	36
	Medium%	30	30
	High School%	32	14
	High education%	12	6
Blood group	A%	20.40	34
	B%	30	15.90
	O%	48.97	47.72
	AB%	0	2.27

II.1.3.2. Study of socioeconomic and clinic factors

Odds ratio (OR) values for Socioeconomic factors (table 03) and Clinicopathological factors (table 04) show that Passive Smoke, Chronic diseases, Contraceptive Pill and Radiation exposure are shown to be significant risk factors for breast cancer (OR = 4.29; p = 0.001, OR=1.976; p=0.0001, OR = 4.15; p = 0.001 and OR = 5.42; p = 0.0001) respectively. Social Problems (OR = 4.69, p = 0.0001) and Sunshine exposed (OR = 2.27, p = 0.035) are also highly significant predictors of breast cancer. In addition, taking Contraceptive pill After the age 30 years, Menopause before 45years, Family history factor and First born After 30 years are all predictive factors (OR ranging from 5.52 to 12.56, p <0.05). Also, Fast Food and Phone in Bras are considered to be very important risk factors in the study population, with the highest OR value (OR = 19.05; p = 0.0001 and OR = 31.06; p = 0.0001). In contrast, Spices and Breastfeeding more than 8 months are protective factors for breast cancer in the study population (OR ranging from 0.279 to 0.444, p <0.036). In addition, our results indicate that Tap water, Cosmetics, Detergents, Puberty After 14 years, PMS Iregular and paracetamol are not considered as predictors of breast cancer in our population since the OR values obtained are not significant.

Table 03: Comparison of the Socioeconomic features of breast cancer patients and control (N=100)

	Control %	Patient %	OR	$CI_{95\%}$	P
Smoke passive			4.292*	1.839-10.017	0.001
Positive	13.26	30.61			
Negative	35.71	20.40			
Fast food			19.056*	2.395-151.598	0.000
Positive	01	14			
Negative	49	36			
Sunshine exposed			2.279*	1.017-5.108	0.035
Positive	17	27			
Negative	33	23			
Chemicals exposed			0.506	0.157-1.635	0.194
Positive	09	05			
Negative	41	45			
Sport			1.536	0.503-4.693	0.318
Negative	42.42	43.43			
Positive	8.08	6.06			
Canned foods			0.900	0.331-2.450	0.520
Positive	10.30	9.27			
Negative	40.20	40.20			
Social Problems			4.696*	1.931-11.418	0.000
Positive	10	27			
Negative	40	23			
Industrial area			5.930	0.667-52.726	0.085

Positive	1.04	4.16			
Negative	51.04	43.75			
Spices			0.444*	0.199-0.989	0.036
Positive	31	21			
Negative	19	29			
Tap water			2.070	0.641-6.686	0.172
Positive	5.10	9.18			
Negative	45.91	39.79			
Obesity			1.833	0.611-5.502	0.207
Positive	06	10			
Negative	44	40			
Phone in Bras			31.069*	3.932-245.472	0.000
Positive	1.29	16.88			
Negative	53.24	28.57			
Detergent			1.397	0.626-3.119	0.270
Positive	28	32			
Negative	22	18			
Sedentarity			3.092*	1.009-9.478	0.037
Positive	5.05	13.13			
Negative	44.44	37.37			
Tight Bras			0.625	0.247-1.584	0.224
Positive	14.14	10.10			
Negative	35.35	40.40			
Cosmetics			0.545	0.239-1.242	0.107
Positive	30	15			
Negative	20	35			

OR>1 and P<0.05 indicate a risk factor
OR<1 and P<0.05 indicate a protective factor

Table 04: Comparison of the Clinicopathological features of breast cancer patients and controls (N=100)

	Control %	Patient %	OR	$CI_{95\%}$	P
Breastfeeding			0.521	0.046-5.942	0.523
Negative	2.24	1.12			
Positive	49.43	47.19			
Breastfeeding more than 8 Ms			0.279*	1.078-11.915	0.027
Positive	43.42	39.47			
Negative	3.94	13.15			
Contraceptive pill			4.156*	1.801-9.587	0.001
Positive	18.08	32.97			
Negative	34.04	14.89			
Contraceptive pill After 30 y			10.907*	3.446-34.525	0.000
Positive	4.16	37.5			
Negative	31.25	27.02			
Puberty after 14 y			0.843	0.322-2.208	0.459
Positive	10.63	9.57			
Negative	38.29	41.48			
PMS Iregular			0.299	0.057-1.559	0.128

Positive	6.06	2.02			
Negative	43.43	48.48			
Menopause Befor 45y			6.864*	2.178-21.636	0.000
Positive	3.84	30.76			
Negative	30.76	34.61			
First born After 30 y			12.564*	1.541-102.417	0.003
Positive	1.14	10.34			
Negative	49.42	39.08			
Childbearing Less 1 year			1.904	1.579-2.296	0.530
Positive	0	1.26			
Negative	46.83	51.89			
Radiation exposure			5.429*	2.302-12.799	0.000
Positive	16.12	37.63			
Negative	32.25	13.97			
Using paracitamol			2.158	0.916-5.087	0.059
Positive	12.24	21.42			
Negative	36.73	29.59			
Family history			5.527*	1.523-20.053	0.004
Positive	2.70	22.97			
Negative	31.08	43.24			
Chronic diseases			1.979*	1.593-2.450	0.000
Positive	0	17.85			
Negative	40.24	41.46			

OR>1 and P<0.05 indicate a risk factor
OR<1 and P<0.05 indicate a protective factor

II.2. Biological markers study

II.2.1. Oxidative Stress markers

According to the result of the table 05, the analysis of the oxidative stress status reveals are a significant increase of GSH concentration in leukocytes ($p < 0.01$), in saliva ($p < 0,05$) and erythrocytes ($p < 0.001$) and of serum ORAC ($p < 0.001$). However, our results show that no significant difference ($P > 0.05$) of MDA level in Leukocyte, erythrocytes and Saliva and catalase activity in leukocytes, and Saliva of the patients group compared to the control.

Table 05: Reduce glutathione (GSH) concentration, Malondialdehyde (MDA) level and catalase activity in Leukocytes, Erythrocytes, saliva and serum ORAC in patients and control groups.

Parameter		Control N=20	Patients N=16	p
Leukocyte	GSH (umol/mgHB)	2.261±0.480	17.96±5.11	0.008
	MDA (nmol/mg HB)	106.74±8.34	107.5±19.3	0.968
	Catalase (UI/g HB)	3.884±0.959	5.37±1.50	0.435
	GSH (umol/mgHB)	8.528±0.925	16.48±1.53	0.000

Erythrocytes	MDA (nmol/mg HB)	16.94±1.69	14.71±1.58	0.520
Saliva	GSH (mmol/mg Pr)	0.425±154	0.844±0.197	0.046
	MDA (umol/mg Pr)	9.95±1.50	8.11±1.20	0.159
	Catalase (UI/mg Pr)	2.231±0.518	2.93±1.36	0.622
Serum	ORAC(UI)	12.020±0.913	15.619±0.542	0.000

The results are presented by mean± SEM.

II.2.2. Biochemical markers

Our results obtained concerning biochemical parameters revealed that a significant increase ($P < 0.05$) in blood glucose level and a significant decrease ($P < 0.01$) in creatinine concentration in women with breast cancer compared to controls. Noting that the changes did not go outside the reference values of the two tests.

Also, our results show that there is no significant change ($P > 0.05$) concerning the serum lipid profile, urea and bilirubin parameters in the groups studied.

Table 06: Mean glucose level and biochemical markers in control and patient groups.

Parameters	Reference values	Control N=20	Patients N=16	P
Glucose (g/l)	0.70-1.10	0.7865±0.0308	1.093±0.121	0.024
Urea (g/l)	0.15-0.50	0.1920±0.0115	0.1925±0.0151	0.974
Creatinine (mg/l)	6-11	6.700±0.363	5.875±0.256	0.006
Total bilirubin (mg/l)	03-12	7.800±0.287	7,067±0,384	0,077
Direct bilirubin (mg/l)	0-4	2.450±0.185	2,533±0,274	0,765
Cholesterol (g/l)	1.2-2.0	1.8330±0.0957	1.916±0.126	0.522
Triglyceride (g/l)	0.5-1.5	0.896±0.186	1.048±0.173	0.393
HDL cholesterol (g/l)	>0.5	0.4906±0.0323	0.4520±0.0319	0.246
LDL cholesterol (g/l)	<1.3	1.1900±0.0950	1.205±0.113	0.899
VLDL (g/l)	0.1-0.3	0.1715±0.0374	0.2096±0.0346	0.287

Values are mean± SEM.

II.2.3. Enzymatic markers

Regarding the enzymatic activity, the results obtained show that no significant variation ($P < 0.05$) of alkaline phosphatase, amylase and transaminases activities in patients compared to the control (table07)

Table 07: Serum enzymes activities in patients and control groups.

Parameters	Reference values	Control N=20	Patients N=16	P
Alkaline phosphatase (UI/l)	50-300	57.60 ±3.23	62.27±7.19	0.527
Amylase (UI/l)	10-90	44.70±3.53	36.33±4.61	0,091
GPT (UI/l)	5-40	24.85±3.38	29.31±5.94	0,464
GOT (UI/l)	5-40	24.65±2.01	48.4±20.9	0,274

Values are mean± SEM.

II.2.4. Metal and electrolytes levels

The result of blood electrolyte and metal analysis (table 08) shows that serum potassium concentration is significant decrease ($P<0.01$) and serum calcium level is significant increase ($P<0.01$) in the breast cancer group against the control. But the changes did not go outside the reference values of the two tests. On the other hand, there were no significant change in serum Na, Cl and iron concentration.

Table 08: Serum electrolyte, iron and calcium level in patients group and controls.

Parameters	Reference values	Control N=20	Patients N=16	P
Na (mmol/l)	135-145	140.92± 1.15	139.84±0.66	0,119
K (mmol/l)	3.5-5	4.714±0.134	4.214±0.131**	0,002
Cl (mmol/l)	95-105	105.69±1.39	104.41±0.79	0,130
Calcium (mg/l)	85-105	83.50±1.81	90.38±2.19**	0,007
Serum iron (mg/l)	0.6-1.7	0.772±0.074	0.804±0.081	0,700

Values are mean± SEM.

II.2.5. Hematological markers

Seen from Table 09 Results of the hematological analysis show that the erythrocyte line (RBC, HGB, HCT) is significantly decreased ($p <0.001$; $p <0.01$ and $p <0.05$) respectively in patients group as the control group and the reference values. Leukocyte lineage (WBC, Neutrophils and basophils), show that no significant differences ($P>0.05$) in breast cancerous women compared to the women controls, Moreover our results illustrate that a significant elevation ($p <0.01$) of monocyte, and a significant decrease of lymphocyte ($p <0.01$) and eosinophil ($p <0.001$), in the patients to the controls. Platelet line, also show that the platelet is significantly increased ($P <0.05$) in cancerous women than the women control.

Table 09: Hematological parameters in the blood of control women and cancerous women.

Parameters	Reference values	Control N=20	Patients N=16	P
White globule cell (10^3/ul)	3.98-10.04	6.472±0.324	5.917±0.613	0,379
Red blood cell (10^6/ul)	3.93-5.22	4.220±0.0674	3.628±0.131***	0,000
Hemoglobin (g/dl)	11.2-15.7	11.320±0.295	10.013±0.364**	0,003
Hematocrit (%)	34.1- 44.9	35.245±0.839	31.44±1.01**	0,002
Booklet (10^3/ul)	182-369	156.6±12.1	225.9±30.4*	0,038
Neutrophil (10^3/ul)	1.56-6.13	3.874±0.306	3.827±0.558	0,934
Lymphocytes (10^3/ul)	1.18-3.74	2.110±0.179	1.515±0.161**	0,002
Monocytes (10^3/ul)	0.24-0.36	0.360±0.033	0.546±0.060**	0,008
Eosinophyle (10^3/ul)	0.24-0.36	0.212±0.357	0.680±0.0153***	0,000
Basophyle (10^3/ul)	0.01-0.08	0.021±0.006	0.020±0.004	0,802

Values are mean± SEM.

II.2.6. Zinc and lead level

II.2.6.1.Zinc level in serum and hair

The results presented in Figure 15 clearly show that the level of Zn in hair and serum patients is lower than the controls.

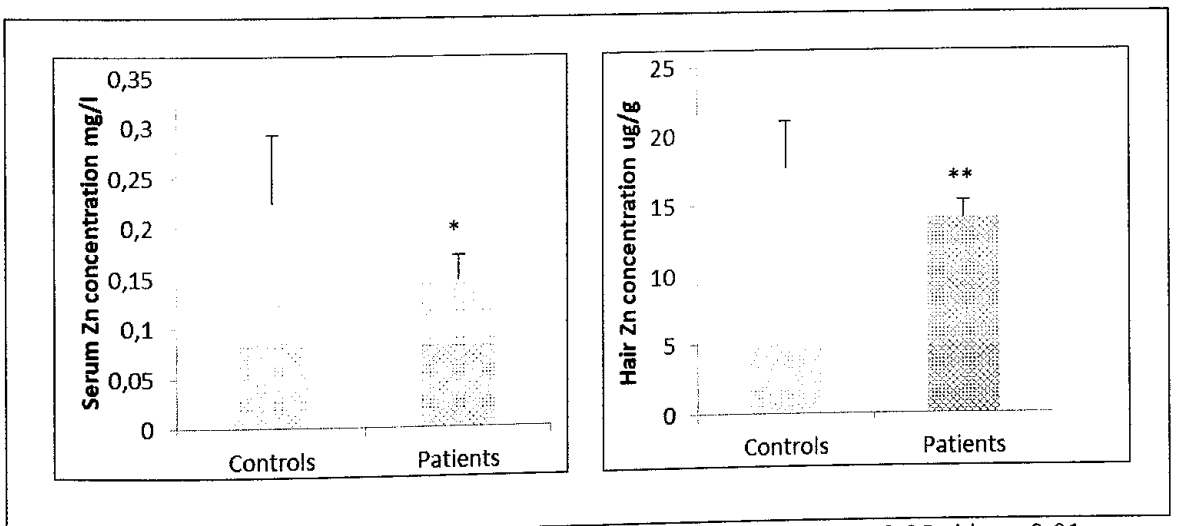


Figure 15: Serum and hair Zn level in control and patients. *: $p<0.05$; **: $p<0.01$.

Table 09: Hematological parameters in the blood of control women and cancerous women.

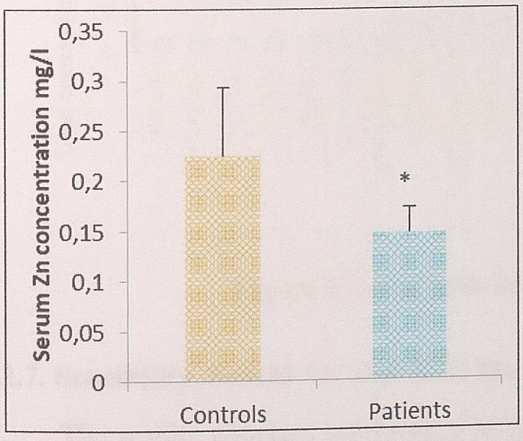
Parameters	Reference values	Control N=20	Patients N=16	P
White globule cell (10^3/ul)	3.98-10.04	6.472±0.324	5.917±0.613	0,379
Red blood cell (10^6/ul)	3.93-5.22	4.220±0.0674	3.628±0.131***	0,000
Hemoglobin (g/dl)	11.2-15.7	11.320±0.295	10.013±0.364**	0,003
Hematocrit (%)	34.1- 44.9	35.245±0.839	31.44±1.01**	0,002
Booklet (10^3/ul)	182-369	156.6±12.1	225.9±30.4*	0,038
Neutrophil (10^3/ul)	1.56-6.13	3.874±0.306	3.827±0.558	0,934
Lymphocytes (10^3/ul)	1.18-3.74	2.110±0.179	1.515±0.161**	0,002
Monocytes (10^3/ul)	0.24-0.36	0.360±0.033	0.546±0.060**	0,008
Eosinophyle (10^3/ul)	0.24-0.36	0.212±0.357	0.680±0.0153***	0,000
Basophyle (10^3/ul)	0.01-0.08	0.021±0.006	0.020±0.004	0,802

Values are mean± SEM.

II.2.6. Zinc and lead level

II.2.6.1.Zinc level in serum and hair

The results presented in Figure 15 clearly show that the level of Zn in hair and serum patients is lower than the controls.



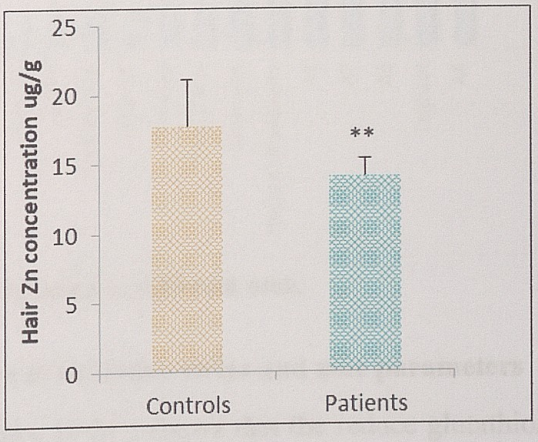


Figure 15: Serum and hair Zn level in control and patients. *: $p<0.05$; **: $p<0.01$.

II.2.6.2. Lead level in serum and soil

The results in Figure 16 presented the level of Pb in serum controls and patients; the result clearly shows that the level of Pb in patients is higher than the controls about 6.67mg/l.

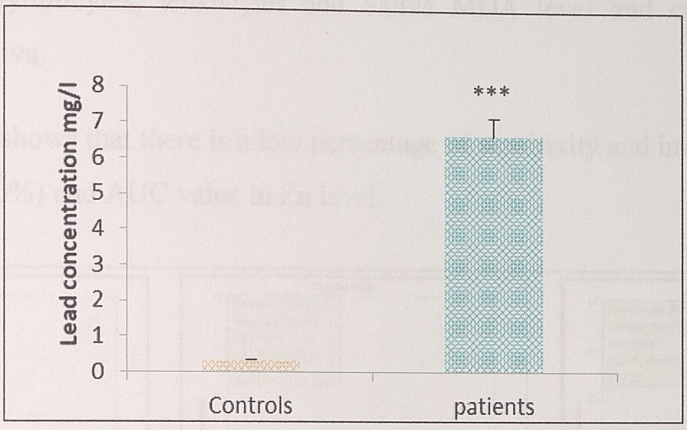


Figure 16: Serum Pb level in control and patients. ***: $p<0.01$

In Figure 17, clarify the result the Pb level, Our result shows that the higher Pb level was determined in Neighboring areas (A4) of El Oued. However, in region of our study, the higher level was in Taghzot with concentration 16.25 mg/kg and the lowest one is Bayada with 0.78m g/kg.

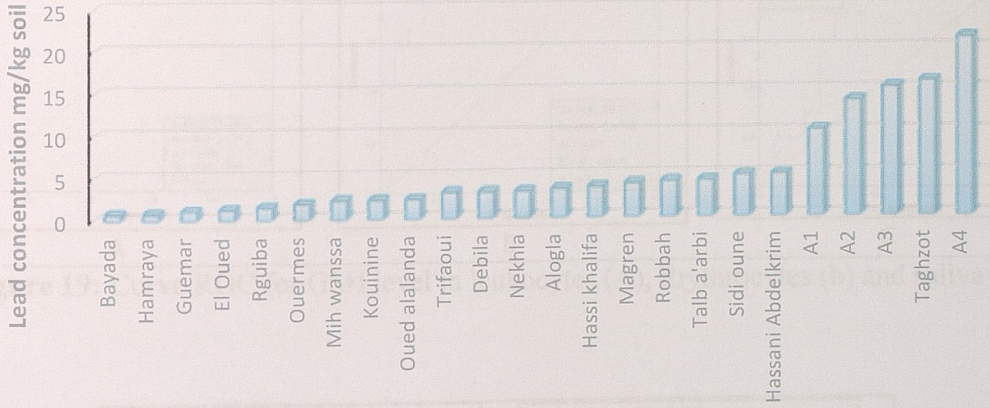


Figure 17: Soil lead concentration in different area.

II.2.7. Sensibility, Specificity and AUC factors of oxidative stress and zinc parameters

The results obtained represented in figures 18-21 ; shows that the reduce glutathione (GSH) level in erythrocytes, leukocytes and Saliva sample and ORAC level in serum stand out as the highest percentage of sensitivity (100, 85.7, 60, 91.7%) with AUC value (0.93,

0.87, 0.732 and 0.823) and important percentage of specificity (63.2, 50, 73.7 and 40%) respectively.

Also, our results show that there is a law percentage of sensitivity and specificity and AUC value in erythrocytes, leukocytes and Saliva MDA level and catalase activity in leukocytes and saliva.

The result shows that there is a low percentage of sensitivity and important percentage of specificity (75.0%) and AUC value in Zn level.

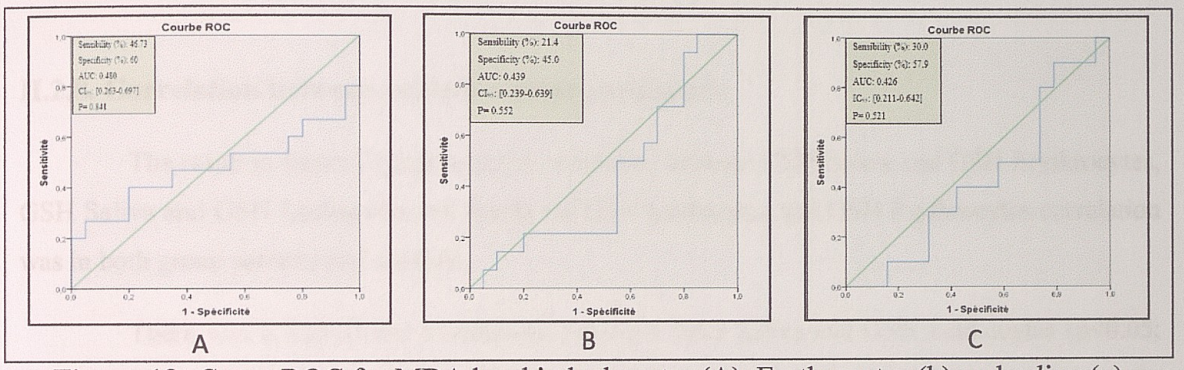


Figure 18: Curve ROC for MDA level in leukocytes (A), Erythrocytes (b) and saliva (c)

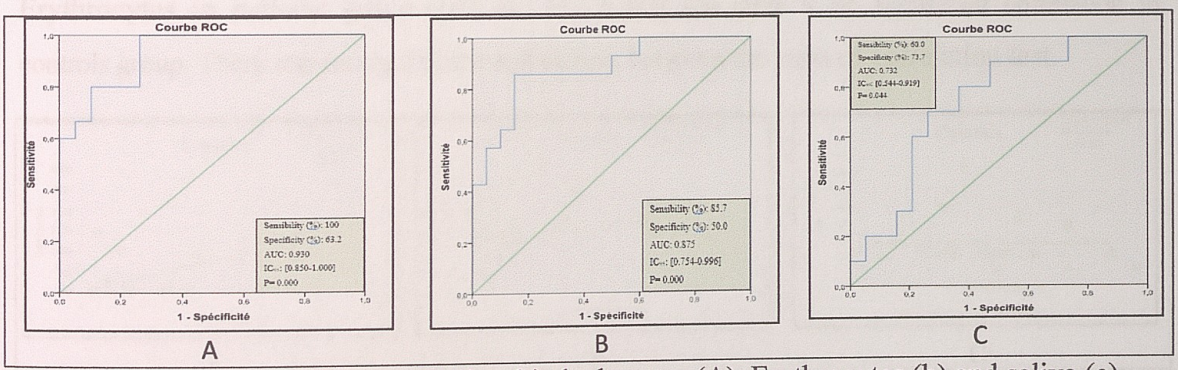


Figure 19: Curve ROC for GSH level in leukocytes (A), Erythrocytes (b) and saliva (c)

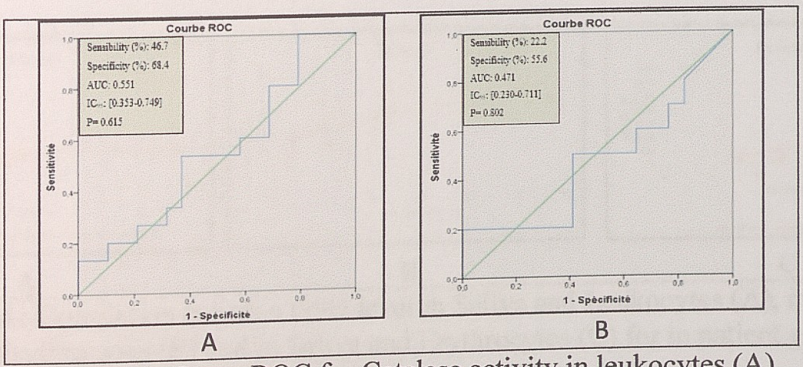


Figure 20: Curve ROC for Catalase activity in leukocytes (A) and saliva (B)

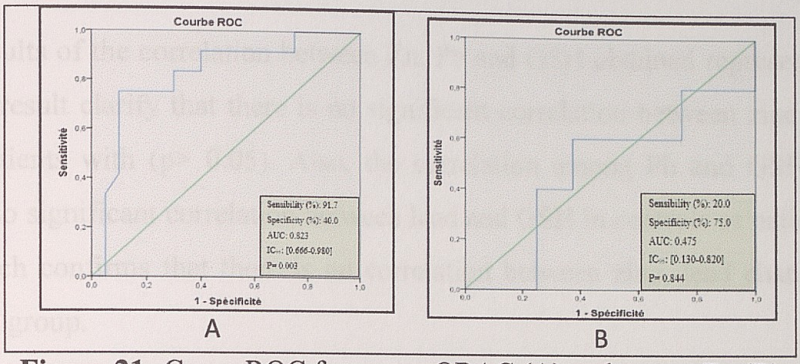


Figure 21: Curve ROC for serum ORAC (A) and serum Zn (B)

II.2.8. Correlation between oxidative stress parameters

The result in figure 28 represent the correlation between GSH Saliva and GSH Erythrocytes, GSH Saliva and GSH Leukocytes and finally the GSH Leukocytes and GSH Erythrocytes correlation was in both group patients and controls.

There was a significant correlation between GSH Saliva and GSH Leukocytes ($p<0.05$; $R^2= 0.255$) in controls group figure **A** while there is no significant correlation in patients group. In addition, there is a significant correlation between correlation GSH Leukocytes and Erythrocytes in patients group ($p<0.05$; $R^2= 0.446$) but there is no significant correlation in controls group. There was no significant correlation between the rests of correlation test.

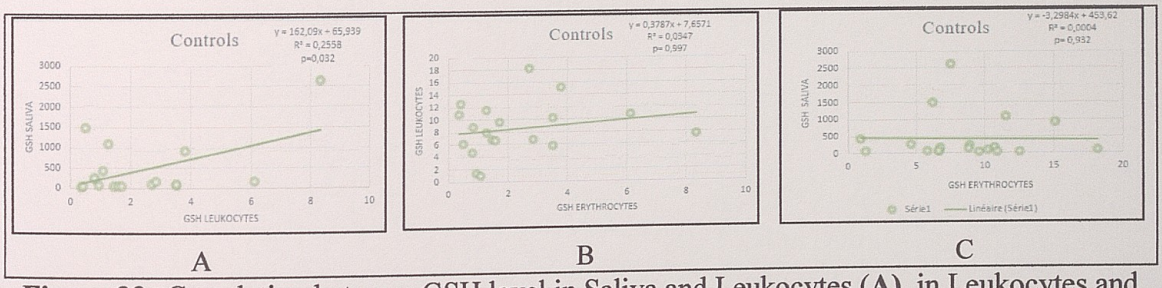


Figure 22: Correlation between GSH level in Saliva and Leukocytes (**A**), in Leukocytes and Erythrocytes (**B**) and in Saliva and Erythrocytes (**C**) for control groups

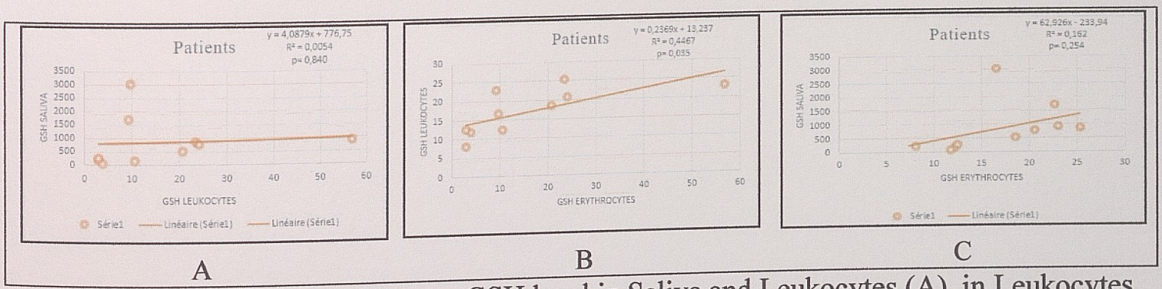


Figure 23: A: Correlation between GSH level in Saliva and Leukocytes (A), in Leukocytes and Erythrocytes (B) and in Saliva and Erythrocytes (C) for in patient groups

II.2.9. Correlation between Zn, Pb and GSH

The results of the correlation between Zn, Pb and GSH obtained represented in tables 10 above, the result clarify that there is no significant correlation between zinc and GSH in controls or patients with ($p > 0.05$). Also, the correlation among Pb and GSH, the results shows that is no significant correlation between lead and GSH in controls or patients with ($p > 0.05$) and which confirms that there is no correlation between zinc, lead change and GSH change in both group.

Table 10: Correlation between Zn, Pb and GSH

Correlation		R^2		P	
		Control	Patient	Control	Patient
Serum Pb	GSH Leukocytes	0.021	0.235	0.430	0.531
	GSH Erythrocytes	0.052	0.032	0.808	0.620
	GSH Saliva	0.058	0.2015	0.057	0.231
Serum Zn	GSH Leukocytes	0.0042	0.3408	0.879	0.502
	GSH Erythrocytes	0.0006	0.4559	0.955	0.749
	GSH Saliva	0.0949	0.1233	0.458	0.896

Discussion

III. Discussion

III.1. Epidemiological study of cancer in the El-Oued region

Breast cancer is one of the dangerous diseases. It occurs in both males and females but the incidence is more in females (Pelumi et al., 2017). In our statistical results men are also at risk of developing breast cancer it was verified too by (Ali, 2017) study, male breast cancer is a rare malignancy that accounts for less than 1% of all cancers in men and less than 1% of all breast cancers. The breast tissues of males and females are identical from birth until puberty when hormonal differences lead to differentiation.

A diagrammatic representation of the age of the patients shows that the majority of breast cancer patients aged between 41-50 years old. Which was found also in other study of (Anders et al., 2009) who shows that the incidence of breast cancer appears to have a sigmoid function in women less than 55 years of age.

Our statistical study show that the left breast is more effected compared to the right one, this is consistent with study of (Tulinius, 1990) who show that the location of cancer in the left breast more than the right one or in the both breasts.

III.2. Study of predictors factors of breast cancer

In this study, we investigated the association between some risk factors and breast cancer.

Passive smoking is the inhalation of smoke. Called second-hand smoke (SHS), or environmental tobacco smoke (ETS), by persons other than the intended "active" smoker. It occurs when Cigarette smoke (CS) permeates any environment, causing its inhalation by people within that environment. Passive smoking contains largely the same components as mainstream smoke, but with varying concentrations (Liuen & Di, 2012). Our study show that the passive smoking is associated with risk having breast cancer, especially if the exposure is long or begins before the first pregnancy, suggesting that the effect of smoking may vary over the course of life and the onset of exposure (Inserm, 2008). In 2005, a pooled analysis of 20 studies that measured both active and passive tobacco exposure was conducted. This study was able to show an increased risk of breast cancer in women who were exposed directly or indirectly to cigarette smoke compared with women who had never been exposed (Johnson, 2005).

In our study the results shows that the eating of fast food, overweight and inactive women was significantly associated with breast cancer risk, other study proved that there is a

strong relationship between this three risk factor (Bassam et al., 2017). Epidemiological evidence shows some discrepancies related to the association between high fat diets and increased risk of tumors (Raúl et al., 2013); Eating "fast food" has increased and linked to obesity (Robert et al., 2006); Obesity is a global problem, affects more than one billion individuals (WHO, 2012). Obesity has been linked to various health disorders, including breast cancer (Alokail et al., 2013). Also known to activate NF-B are several cytokines produced by adipocytes, such as leptin, tumor necrosis factor (TNF), and interleukin-1 (IL-1) (Preetha et al., 2008). (Hildebrand et al., 2013) showed that a greater reduction in risk is associated with increasing amounts of exercise and more vigorous activity. however, even smaller amounts of exercise, including walking, appear beneficial. Also, the most accomplished studies about effect of physical activity on the breast cancer risk demonstrate that inactive women have a higher risk of the breast cancer compared to physically active women (Pizot et al, 2016; Kyu et al., 2016).

Our study showed that psychological stress is risk factor for breast cancer. Study of Mei-Ling and Tso-Ying (2015) showed that anxiety, depression, and stress might tentatively be related to breast cancer incidence. Psychosocial factors such as personality traits and depression may alter immune and endocrine function, with possible effects on cancer incidence (Naoki, 2014). Stress-related immune changes will highlight natural killer (NK) cells, because of their importance for cancer. NK cells play an important role in a variety of immune functions, including defense against viral infections and surveillance of tumor cells. There is evidence that stress can also inhibit the response of NK cells (Kiecolt-Glaser & Glaser, 1999). Also stressors involve central nervous system (CNS) perceptions of threat and subsequent activation of the hypothalamic–pituitary– adrenal (HPA) axis. Catecholamines, glucocorticoids and other stress hormones are subsequently released from the adrenal gland, Stress hormones can also activate oncogenic viruses and alter several aspects of immune function (Michael et al., 2006).

The ozone layer is a layer in earth's atmosphere which contains relatively high concentrations of ozone (O_3). This layer absorbs 93-99% of the sun's high frequency ultraviolet light, which is potentially damaging to life on earth (Sivasakthivel & Siva Kumar Reddy, 2011). Therefore; UV has complex and mixed effects on human health. Nonetheless, excessive exposure to UV carries profound health risks, including atrophy, pigmentary changes, wrinkling and malignancy (John et al., 2013).UV-B radiation can adversely affect the immune system causing a number of infectious diseases (Sivasakthivel & Siva Kumar Reddy, 2011).

Our study shows that sun radiation (UV) exposure is a risk factor associated with breast cancer. Ultraviolet A (UVA) photons trigger oxidative reactions by excitation of endogenous photosensitizers, such as porphyrins, NADPH oxidase, and riboflavins. 8-Oxo-7,8- dihydroguanine (8-oxoGua) is the main UVA-mediated DNA oxidation product formed by the oxidation of OH radical, 1-electron oxidants, and singlet oxygen that mainly reacts with guanine (Esra et al., 2012).

In our result showed that women kept their cell phones in their bra is risk factor; chronic exposure to radiofrequency electromagnetic radiation of cell phone leads to that is associated with increased oxidative stress (Oyewopo, 2017) because the soft fat breast tissue readily absorbs such radiation. In addition, a study conducted at the University of California reported. However, these women all regularly kept their cell phones in their bra adjacent to their breast during the day, which was estimated to be about 10 hours every day for several years. Each presented with tumors in the same regions of their breasts, next to where their phones were kept (Saddig, 2016).

Our study shows that first born after the age 30 years is a risk factor. this result are in agreement with the studies of the (McPherson et al.,2000), who showed the risk of breast cancer in women who have their first child after the age of 30 years is about twice that of women who have their first child before the age of 20 years.

Importantly; our study showed associated for menopause and risk having BC. Only few studies investigated the relationship between BC and menopause. The results found were similar to those observed in (Howell et al., 2014) studies; women who experience menopause at age 55 years or older have about a 12% higher risk compared to those who do so between ages 50-54 year. The increased risk may be due to longer lifetime exposure to reproductive hormones and has been more strongly linked to breast cancer than other subtypes (ACS, 2017). Women who have a natural menopause after the age of 55 years are twice as likely to develop breast cancer as women who experience the menopause before the age of 45 years (McPherson et al., 2000).

In our study it was observed that the radiation is associated with BC, where (Key et al., 2001) explain that the breast is among the tissues that are most sensitive to the effects of radiation. Exposure of the chest of women below the age of 40 years to ionizing radiation is known to increase risk for breast cancer (Jennifer, 1996). Ionizing radiation causes several forms of DNA damage (Alice, 2007).

In our study, consumption of Contraceptives pills is associated with increased risk of breast cancer. Contraceptive use among women with cancer was very important compared to control women. According to Collaborative Group on Hormonal Factor in Breast Cancer (1997), the risk of breast cancer is increased by about 25% in women who commonly use contraceptives pills.

Concerning the age of use Contraceptive pill, our results shows that the use after the age 30 years is a risk factor for obtained breast cancer. The results were disagreed to those observed in (McPherson et al., 2000), showed that Women who begin use before the age of 20 years appear to have a higher relative risk than women who begin oral contraceptive use at an older age.

In our study, we found higher risk of breast cancer in association with the Family history. According to (ACS, 2017), Women and men with a family history of breast cancer especially in a first-degree relative (parent, child, or sibling), are at increased risk for the disease, (Taheripanah et al., 2018), Approved that there was significantly different between the groups was the positive family history of breast cancer.

Genetic linkage studies in multiple-breast cancer families have identified the BRCA1 and BRCA2 genes that are tumor suppressor genes (Chompret; 2003). Mutations in high-risk breast cancer genes such as BRCA1/2 affect only small numbers of women (Howell et al., 2014). Their main function is to preserve the chromosomal structure through their involvement in DNA repair processes and recombination, cell cycle control and transcription of other genes. (Venkitaraman, 2002). Between 5 and 10% of all breast and ovarian cancers are thought to be the consequence of susceptibility genes, of which 2 to 3% are caused by BRCA1 and BRCA2. It is therefore a rare situation (Chompret; 2003).

The presence of one or more chronic diseases may make receipt of cancer screening even more complex. Some chronic illnesses, such as diabetes, serve as independent risk factors for certain cancers (Michels et al., 2003) and may be associated with cancer mortality (Coughlin et al., 2004). Other studies have found the presence of chronic diseases is associated with better cancer screening utilization. Patients with hypertension have been reported to have more breast exams (Betty et al., 2014).

Our results showed that the patients with concomitant diseases such as diabetes and hypertension associated with developing breast cancer.

The diabetic state leads to hormonal changes such as increased concentrations of insulin (Ana et al., 2014), decreased concentrations of sex hormone-binding globulin, and

increased concentrations of estradiol (Xue & Michels, 2007; Onitilo et al., 2012). Furthermore, the mitogenic properties of insulin are well known (Chappell et al., 2001) and similar to the proliferative effects of leptin on cancer cells, which is highly expressed in obese patients (Maccio & Madeddu, 2011; Hu et al., 2002). Although an association is plausible, published results on the link between diabetes and breast cancer have been variable (Ana et al., 2014; Liao et al., 2011). Conducted a meta-analysis of16 studies published between 2000 and 2010, the combined results detected a 23% increase in the risk of breast cancer in women with diabetes (Gertraud et al., 2017; Ana et al., 2014)

Hypertension, a common chronic disease and major risk factor for breast cancer (Staessen et al., 2003). According to (Hedong et al., 2017) the results suggested a statistically significant 15% increase in risk of breast cancer.

In our study population breastfeeding more than 8 months are protective factors from breast cancer, According to (Collaborative, 2002) The effect of breastfeeding on the risk of breast cancer is controversial; women who have breastfed for a total of at least 25 months present a 33% reduced risk compared to those who never breastfed. A significant reduction in breast cancer risk of more than 4% has been reported for each 12-month breastfeeding period (Collaborative, 2002). In general, the longer the duration of breastfeeding, the more women are protected against breast cancer. (Jessica et al., 2013). Lactation produces endogenous hormonal changes, in particular a reduction of estrogens (Nkondjock & Ghadirian, 2005) that play a regulatory role or stimulation of cancerous proliferation. Prolonged exposure of breast tissue to the hyperoestrogenicpeaks of the hormonal cycle appears to be the most important factor (Puddu & Tafforeau, 2005). Lactation increase in prolactin production, which is believed to decrease cumulative estrogen exposure in women. Therefore, lactation would suppress the onset and development of breast cancer (Key et al., 1988).

Our results showed that the spices are protective factors from breast cancer, According to (Jie et al., 2016) Spices have been widely used as food flavorings and folk medicines for thousands of years. Numerous studies have documented the antioxidant, anti-inflammatory and immunemodulatory effects of spices, which might be related to prevention and treatment of several cancers, including breast cancers.

III.3. Biological markers study

Our study showed that the RBC, HGB, HCT are significantly decreased in the breast patients as compared to control. Our results are in agreement with the results of (Shrivastava et al., 2017) that got that found a significantly lower of hemoglobin level and RBC count in women with breast cancer as compared to control healthy subjects. Moreover our results illustrate that a significant elevation monocyte, and a decrease of lymphocyte and eosinophil, in the patients to the controls. (Khan et al., 2017) also found the percentage of monocytes and lymphocytes were significantly in present study. (Iqbal et al.2015) showed also that decrease in eosinophils numbers, in cancer patients when compared with healthy individuals. Platelet line, also show that the platelet is significantly increased in cancerous women than the women control. The results found were opposite to those observed in (Shrivastava et al., 2017) who showed that platelet were non-significant change as compared to control healthy subjects. Variation in hematological parameters in breast cancer patients may be due to the increase in the levels of pro-inflammatory cytokines including Interlukin-I and Interlukin-6 and Tumor necrosis factor (Khan et al., 2017).

The role of trace elements in breast cancer development has been investigated but remains to be elucidated. We sought to see if there were systemic differences in serum levels of trace elements between breast cancer patients and matched controls. In our study, compared to controls, serum Zn levels had significantly low in breast cancer patients which agreemen with result of HONG et al., 2006 study, other Epidemiological studies indicated that content of Zn in serum of tumor patients was lower than in healthy persons (Ateeqet al., 2006). Hair may serve as a good biopsy material for assessment of trace elements status, In other types of tumours, hair zinc levels were signicantly lower in tumour patients than in controls (Wu et al., 2015). Our study also found that there was a lower hair zinc level in the breast cancer patients, which supports the hypothesis that breast cancer cells consume more zinc, thus reducing hair zinc levels (Wu et al., 2015).

Zinc is a trace mineral which requirement for all life on earth, despite being the 27th most abundant element, the physiological importance of zinc is unparalleled in its functional diversity (Stipanuk & Falchuk, 1993). Zinc plays an important role in normal mammary gland growth and development and during lactation and post-lactation transformations (McCormick et al., 2014). It has been recognized as a critical signaling molecule in normal cell physiology as well as in pathophysiological conditions, such as cancer (Liang et al., 2016). Under normal conditions, zinc has been shown to regulate several phosphorylation-dependent signaling cascades, including MAPKs and Akt, that play important roles in cell development,

proliferation and cell death. Many of these same signaling cascades have been shown to be affected by zinc levels in cancer and lead to cancer cell proliferation and metastasis (Bafaro et al., 2017)

In our study, breast cancer patients had significantly high serum Pb levels compared to controls, this result versus the result of (Xiao et el., 2014) who found that there is no significant difference in Pb level compared to controls.

People are exposed to lead mainly by breathing it (from dust or fumes) or swallowing it. Because of its widespread use over the years, exposure to lead in the environment is now more likely to come from man-made rather than natural sources. Lead can change forms (for example from organic to inorganic) and can move around in the environment (for example, from the air to the soil to the water), but it does not break down and go away (IARC, 2014).

There is limited evidence for the carcinogenicity to humans following exposure to inorganic lead compounds. In considering the genetic and related effects of exposure to lead, the Working Group discussed the mechanistic aspects of lead as a potential carcinogen. They concluded that there is little evidence that lead interacts directly with DNA (PEH, 2017). lead-induced alteration in gene expression appear to be mediated in part by the modulation of reactive oxygen species and the interaction with proteins, including those involved in DNA repair, which may contribute to a carcinogenic response if exposure is sustained (Patra et al., 2011).

GSH exhibit multidirectional role in cellular processes including cell growth, apoptosis, defense against oxidative stress as well as in chemo resistance and progression of cancer (Javed et al., 2015).

In our experimental study, the results show a significant increase in glutathione levels, this result are agreement with the study of (Javed et al., 2015). Who showed a significantly increased level of GSH was observed in breast cancer patient. A positive correlation has been observed between increased GSH synthesis and high levels of cell proliferation in tumors (Obrador et al., 1997). Rise level of GSH support metastatic invasions of cancer (Nicola et al., 2013).

The Oxygen Radical Absorbance Capacity (ORAC) is used determined the antioxidant potential (Viduranga & Lee, 2013). In our experimental study, our data revealed that the total antioxidant activity (ORAC) was increased in the serum of breast cancer patients than in control women. The results found were opposite to those observed in (Badid et al., 2009); showed that ORAC alues were lower in breast cancer patients than in control women.

During oxidative metabolism harmful reactive oxygen species (ROS) are generated. These species are neutralized by antioxidant enzymes (Haan et al., 1995). Superoxide dismutase (SOD) is the first detoxification enzyme and most powerful antioxidant in the cell. It is an important endogenous antioxidant enzyme that acts as a component of first line defense system against reactive oxygen species (ROS). The metal ions which are normally bound by SOD is zinc (Zn) (Ighodaro & Akinloye, 2017).

SOD converts superoxide radicals ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2) while Glutathione peroxidase (GPx) independently convert this to water (Haan et al., 1995). using electrons from the glutathione (GSH) (Mladenov et al., 2015). The GPX enzymes oxidize the thiolcontaining peptide glutathione (GSH) to its oxidized form, glutathione disulfide (GSSG), using H_2O_2 as the electron donor (Fabio et al., 2016).

Investigation of Zn-SOD activities revealed that the lower SOD activity was because of decreased Zn level (Raeve et al., 1997). A decrease in GPx increases GSH concentration, the negative correlation between GSH and GPx seems to be clear (Lukaszewicz & Moniuszko, 2003). An imbalance in the ratio of Sod to Gpx results in the accumulation of H2O2 which may participate in the Fenton reaction, resulting in the formation of noxious hydroxyl radicals (Haan et al., 1995).

Conclusion and Prospects

Conclusion

Breast cancer, is a most common cancer worldwide, accounts for the highest mortality. We are therefore important to have a diagnostic tool as early as possible, thus possibly improving the prognosis. Several risk factors have been studied to find out which one is really involved in carcinogenesis.

The results of our study showed that breast cancer disease is distributed in the different communes of El-Oued state with more frequent in women approximately 98% than men. Where the most age group to touch are [41-50] years. We observed that the left breast is more affected with frequency 52% compared to the right breast.

The results of our study showed that Passive Smoke, Contraceptive Pill, Radiation Exposure, Social Problems, Sunshine exposed, Contraceptive pill After 30 years, Menopause Befor 45years, First born After 30 years, Fast Food, Phone in Bras are shown to be majors risk factors for breast cancer, which indicates the importance of social behavior and the clinical factor of breast cancer involvement. In contrast, Spices and Breastfeeding more than8 Months are protective factors for breast cancer in the study population. In addition, our results indicate that Tap water, Cosmetics, detergents, Puberty after 14 years, PMS Irregular and paracetamol are not considered as predictors factors of breast cancer in our population.

Our results show that cancerous women show no variation in biochemistry and enzymatic parameters except creatinine which directly affects the energy level at the muscular level. Therefore our results indicate biochemistry parameters and enzymatic are not specific markers for diagnostic of the breast cancer. Moreover, we observed in our work a variation of hematological markers such as the decrease of hemoglobin, Hematocrit and red blood cell and lymphocyte and eosinophil. This Decrease of the red blood cell rate causes oxygen transport disturbance which favors the appearance of the other complication and that hematological parameters are an important markers of diagnosis and therapeutic follow-up of the disease. Our work also reveals monocyte and platelet rate increases. These elevations characterized by the presence of inflammatory case.

Results demonstrated the high lead level in serum and soil. This study revaluated environmental contamination by lead is a very important factor for breast cancer expected in the region of El Oued. Moreover, we obtained also the low Zinc level in serum and hair of breast cancer patients. This result indicates the importance of diet factors and zinc deficiency in breast cancer expected with no relative effect on stress oxidative markers.

Our work revealed a variation of GSH and antioxidant total (ORAC) that indicates there is a variation of antioxidant defense system derived from a high activity of oxidative

stress that has caused a disturbance of oxidative / antioxidant status. These alterations are due to the fact that breast cancer is a case pathology progresses and initialized by oxidative stress.

On the other hand our result shows that the reduce glutathione (GSH) level in Erythrocytes and serum ORAC represented the highest percentage of sensitivity and important percentage of specificity and AUC. Therefore, our results indicate this parameter is new reliable marker for diagnostic and predictive against breast cancer in women.

Prospects

Given the importance of these results, they open experimental perspectives and other in-depth studies that should allow us to clearly identify:

- Determine genetic polymorphism in women with breast cancer.
- Comparison of the population of region El Oued with other region.
- Determine other factors associated with breast cancer risk.
- Evaluation of diagnostic marker variation in cancerous women and women healing.

Bibliographical references

Bibliographical references

1. Aebi H. (1984). catalase invitro. Meth.enzymol, 105.121_126.
2. Aftab, A., Saira, A., Rabail, H.T., Abdul, RS. (2015). Cytotoxic and Genotoxic Effect of Arsenic and Lead on Rat Mesenchymal Stem Cells (rMSCs). Pakistan J. Zool., 47(1), 41-47.
3. Agarwal, A., Banerjee, A., Banerjee, UC. (2011). Xanthine oxidoreductase: a journey from purine metabolism to cardiovascular excitation-contraction coupling. Crit Rev Biotechnol, 31, 264-80.
4. Agency for Toxic Substances and Disease Registry (ATSDR). (2007). Toxicological profile for lead.
5. Aggarwal. (2004). Nuclear factor-kappaB: the enemy within. Cancer Cell, 6:203–208 doi:10.1016/j.ccr.2004.09.003.
6. Ahmadi-Vincu, M., Pup,M., Gârban , G., Ariana-Bianca ,V., Gârban, Z., Velciov,R.P. (2005). Influence of zinc over-doses on some metals in wistar rats brain. Scientifical Researches. Agroalimentary Processes and Technologies, Volume 11 (1), 153-160
7. Akulapalli, S. (2009) History of Cancer, Ancient and Modern Treatment Methods. J Cancer Sci Ther. doi: 10.4172/1948-5956.100000e2
8. Alena, S., Daniela, W., Jitka, V. (2006). Ultraviolet light induced alteration to the skin. Biomedical Papers, 150(1), 25-38.
9. Alfonso, V B., Julio, S., Susana, N. (2003). Natural antioxidants in functional foods: from food safety to health benefits. Grasas y Aceites, 54. (3), 295-303. doi.org/10.3989/gya.2003.245.
10. Ali, J. Y., (2017). Male Breast Cancer: Epidemiology and Risk Factors. Seminars in Oncology, doi:10.1053/j.seminoncol.2017.11.002
11. Alice, J. S., Parveen ,B., Michele, M. D., Michael, H., Laura, B., Steven, L. S., Robert,M. W., Martha,S. L., Marvin, R., Marilyn, S., Bruce, H. A., Dale,L. P., Jeffrey, P.S., Preetha, R. (2007). Polymorphisms in Apoptosis- and Proliferation-Related Genes, Ionizing Radiation Exposure, and Risk of Breast Cancer among U.S. Radiologic Technologists. Cancer Epidemiol Biomarkers Prev, 16(10), 2000-2007.
12. Almeras, C., Bringer-Guerin,C., Clinard,F., Daniau,C., Denys,S., Dor,F., Epelboin, A., Erouart,S., Etchevers,A., Grard,O., Hulot,C., Jehanin,P., Leger,T., Piantone,P., Salvio,C. (2013). Sites potentially polluted by lead: feedback and recommendations Ministry of Social Affairs and Health. (Ed).

13. Alokail, M.S., Al-Daghri, N., Abdulkareem, A. (2013) Metabolic syndrome biomarkers and early breast cancer in Saudi women: evidence for the presence of a systemic stress response and/or a pre-existing metabolic syndrome-related neoplasia risk? BMC Cancer 13 (54), http://dx.doi. org/10.1186/1471-2407-13-54.
14. Alugoju, P., Dinesh, B. J., Latha, P. (2015). Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Ind J Clin Biochem, 30(1):11–26.
15. American Cancer Society (ACS). (2017). Breast Cancer Facts & Figures 2017-2018. Atlanta: American Cancer Society, Inc.
16. Ana, M. S., Lillian, I. F., Juan, M. C., Brenda, H., Alberto, A., Julia V., Eduardo E. G., (2014). Prediabetes, Diabetes, and Risk of Breast Cancer: A Case-Control Study. Archives of Medical Research 45 432e438.
17. Ananda, S. P. (2007). Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. Experimental Gerontology, 43, 370–377. doi:10.1016/j.exger.2007.10.013.
18. Anders, CK., Johnson, R., Litton, J., Phillips, M., Bleyer, A. (2009). Breast Cancer Before Age 40 Years. Seminars in oncology, 36(3):237-249. doi:10.1053/j.seminoncol.2009.03.001.
19. Andrew, M. (2012). Cancer - not my story, ISBN: 978-1-105-74112-8
20. Anna, K., Oleg, B., Gergana, M., Aleksandra, G., Andy, R., Elena M. K., Olga M. P., Ian J. J., Martin, W., Usha, M., John, F. T. (2017). Testing breast cancer serum biomarkers for early detection and prognosis in prediagnosis samples. British Journal of Cancer 116, 501–508; doi:10.1038/bjc.2016.433
21. Anu, R., Amit, K., Vivek, S., Brijesh , Y., Ruchi, T., Sandip, C., Kuldeep, D. (2014). Oxidative Stress, Prooxidants, and Antioxidants: The Interplay. Bio Med Research International, 2014, 19. Doi.org/10.1155/2014/761264.
22. Ashim, K. M., Vibhuti, A., Abhirup, M., Kishore, C., Chandramouli, N., Sujay, S., Mary, J., Hoang, M. T., Ravi V., Xiaoyan, Y., Yi, H., Varun, K., Dhananjay, P., Novel delivery approaches for cancer therapeutics. (2016). J Control Release, 219: 248–268. doi:10.1016/j.jconrel.2015.09.067.
23. Ateeq, R. M., Tasneem G. K., Hassan, I. A., Mohammad., K. J., Mohammad, B. A., Nusrat, J., Nasreen, S. (2007). Evaluation of zinc status in whole blood and scalp hair of female cancer patients. Clinica Chimica Acta, 379 66– 70, doi:10.1016/j.cca.2006.12.009

24. Badid, N., Baba Ahmed , F.Z., Merzouk,H., Belbraouet, S., Mokhtari ,N., Merzouk , S.H., Benhabib , R., Hamzaoui ,D.,Narce,M.(2009). Oxidant/Antioxidant Status, Lipids and Hormonal Profile in Overweight Women with Breast Cancer. Pathol Oncol Res, 16(2),159-67.DOI: 10.1007/s12253-009-9199-0 .

25. Bafaro, E., Liu, Y., Xu, Y0, Dempski, R.E. (2017). The emerging role of zinc transporters in cellular homeostasis and cancer. Signal transduction and targeted therapy, doi:10.1038/sigtrans.2017.29.

26. Balazs, I. B. (2015). FACS; Phillip Tuso, MD, FACP, FASN; Breast Cancer Survivorship: A Comprehensive Review of Long-Term Medical Issues and Lifestyle Recommendations. ORIGINAL RESEARCH & CONTRIBUTIONS, http://dx.doi.org/10.7812/TPP/14-241.

27. Bassam, A. A., Rakan, F. A., Ahmed, A. A., Abdulrhman, M. A., Bandar, T. A., Haitham, S. A., Hussain, G. A. (2017). Breast cancer in Saudi Arabia and its possible risk factors, Journal of Cancer Policy 12 83–89 , http://dx.doi.org/10.1016/j.jcpo.2017.03.004

28. Basu, P., Mittal, S., Bhadra, V. D., Chami, K. Y. (2017). Secondary Prevention of Cervical Cancer. Best Practice & Research Clinical Obstetrics & Gynaecology, doi: 10.1016/j.bpobgyn.2017.08.012.

29. Baynes J.W., Thorpe S. (1999). Role of oxidative stress in diabetic complications; a new prespective on an old paradigm, diabetes, p48:1-9.

30. Bayo, M. A. Castano, F., Rivera, F. N., (2017). Analysis of blood markers for early breast cancer diagnosis; Clin Transl Oncol, DOI 10.1007/s12094-017-1731-1

31. Belpomme, P. I., Hardell, L., Clapp, R., Montagnier, L., Epstein, S., Sasco, A. J. (2007). The multitude and diversity of environmental carcinogens. Environ. Res. 105:414–429, doi:10.1016/j.envres.2007.07.002.

32. Berg, W.A, Blume, J.D, Cormack, J.B, et al. (2008). Combined screening with ultrasound and mammography vs mammography alone in women at elevated risk of breast cancer, JAMA 299(18):2151–63.

33. Bernstein, C.R., Teal, S., Joslyn, J. W. (2003). Ethnicity-related variation in breast cancer risk factors. Cancer 97 222–229, doi: http://dx.doi.org/10.1002/cncr.11014.

34. Bertrand, P., Severine, J., Jerome, S., Eric, P. (2011). Lead-induced DNA damage in Vicia faba root cells: Potential involvement of oxidative stress. Mutation Research - Genetic Toxicology and Environmental Mutagenesis, 726 (2), 123-128. DOI:10.1016/j.mrgentox.2011.09.001.

35. Betty, Y. L., Jean, O., Motomi, M., Lyle, J. F., David, L., Cynthia, D. M., David, I. B., John, D. H., Patricia, A. C. (2014). The Association of Type and Number of Chronic Diseases with Breast, Cervical and Colorectal Cancer Screening in Rural Primary Care Practices; Utah, J Am Board Fam Med.; 27(5): 669–681. doi:10.3122/jabfm.2014.05.140005.
36. Bingham, S. A., Hughes, R., Cross, A. J. (2002). Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. Journal Nutrition. 132:3522S–3525S.
37. BLACHE, D., PROST, M., (1992). Free radical attack: Biological test for human resistance capability. In proceeding of the college park on chemical evolution. Chemical Analyses Laboratory. NASA, WASHINGTON. 82-98.
38. BOICE JD., Cancer following irradiation in childhood and adolescence. Med. Pediatr. Oncol. 1996; 1(suppl.):29-34.
39. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer ; The Lancet, Volume 350, Issue 9084, 1997, Pages 1047-1059 ; https://doi.org/10.1016/S0140-6736(05)64261-4.
40. Bruce, A. F., David, C. B., Robert, L. B., Julian; C.J., Henry, F., Russell, F.A., Kathryn, R. M., Paul, M., John, F. R., Joel, S., Rodney. S., George, P.(1993). Measuring Lead Exposure in Infants, Children, and Other Sensitive Populations. National Academy of Sciences. ISBN: 0-309-59841-9.
41. Bukola, R. O., Adeleke, A..A., Omamuyovwi, M. I., Stephen, O.A. (2015). Lead-Induced Damage on Hepatocytes and Hepatic Reticular Fibres in Rats; Protective Role of Aqueous Extract of Moringaoleifera Leaves (Lam). Journal of Biosciences and Medicines, 3, 27-35 .Doi.org/10.4236/jbm.2015.35004.
42. Canadian Cancer Society, Breast Cancer: Breasts, 2018; http://www.cancer.ca/fr-ca/cancer-information/cancer-type/breast/breast-cancer/the-breasts/?region=qc
43. Cancer et environnement. (2008). Rapport. Paris : Les éditions Inserm, XVII- 889 p. - (Expertise collective) - http://hdl.handle.net/10608/102
44. Canchola, A.J., Anton-Culver, H., Bernstein, L., Clarke, C.A., Henderson, K., et al., (2012). Body size and the risk of postmenopausal breast cancer subtypes in the California teachers study cohort. Cancer Causes Control (January), doi: http://dx.doi.org/10.1007/s10552-012-9897-x.

45. Carla, F., Stefano, G. (2013). Analysis of oxidative and free radical induced dna damage. Doctoral thesis, University Prague.

46. Chappell, J., Leitner, J.W., Solomon, S. (2001) et al. Effect of insulin on cell cycle progression in MCF-7 breast cancer cells. Direct and potentiating influence. J Biol Chem, 276:38023e38028.

47. Cheeseman, K., Cheeseman, K., Rouleau, E., Vannier, A., Thomas, A., Briaux, A., Lefol, C, Walrafen, P., Bensimon, A., Lidereau, R., Conseiller, E., Ceppi, M. (2012). A diagnostic genetic test for the physical mapping of germline rearrangements in the susceptibility breast cancer genes BRCA1 and BRCA2. Hum Mutat, 33(6): p. 998-1009.

48. Chetlen, A., Huesch, M., Segel, J., & Schetter, S. (2017). Frequencies of Private Mentions and Sharing of Mammography and Breast Cancer Terms on Facebook: A Pilot Study. Journal of Medical Internet Research, 19(6), e201. http://doi.org/10.2196/jmir.7508

49. Chompret, (2003). Genetic diagnosis of breast cancer and hereditary ovary; J Gynecol Obstet Biol Reprod; 32 : 101-119.

50. Claeyssen, R., Andriollo-Sanchez, M., Arnaud, J., Touvard, L., Alonso, A., Chancerelle, Y., Roussel, A.M., Agay, D. (2009). Effect of sub-deficient zinc status on insulin sensitivity after burn injury in rats. Biol. Trace Elem. *Res.* 127: 132-42.

51. Clement, G. Y., Hervey, M. T., Paul, B. T. (2015). DNA Damage, Cell Cycle Arrest, and Apoptosis Induction Caused by Lead in Human Leukemia Cells. International Journal of Environmental Research and Public Health, 13(1), 56. Doi: 10.3390/ijerph13010056.

52. Cleveland Clinic Foundation, Breast Cancer in Young Women, 2015 https://my.clevelandclinic.org/health/articles/16805-breast-cancer-in-young-women

53. Collaborative group on hormonal factors in breast cancer. (2002). Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50,302 women with breast cancer and 96,973 women without the disease. 360:187-95.

54. Coughlin, S.S., Calle, E.E., Teras, L.R., Petrelli, J., Thun M.J. (2004). Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. Am J Epidemiol. 159 (12):1160–1167.

55. Daniel. S. M., Priya, B., Christian, L., Jennifer, B., Chelsia, G., Lianne, T., Julie, K. S. (2017). The Case for Prehabilitation Prior to Breast Cancer Treatment ; the American

Academy of Physical Medicine and Rehabilitation, http://dx.doi.org/10.1016/j.pmrj.2017.08.402

56. David, A. J. & Mary, K. G. (2015). Radiation Therapy for Cancer. Cancer: Disease Control Priorities, Third Edition (Volume 3).
57. Denissenko, F., Pao, A., Tang, M., Pfeifer , G.P. (1996). Preferential formation of benzopyrene adducts at lung cancer mutational hotspots in P53. Science. 274:430–2 doi:10.1126/science.274.5286.430.
58. Derouiche, S., Djermoune, M., Abbas, K. (2017b). Beneficial Effect of Zinc on diabetes induced kidney damage and liver stress oxidative in rats. journal of advances in biology. DOI: 10.24297/jab.v10i1.6022.
59. Derouiche, S., Djouadi, A. (2017). Control of Lead and Cadmium in Cosmetic Product (Kohl) of Pits Dates by Cyclic Voltammetry. J. Chem. Pharm. Res., 9(3), 319-323.
60. Derouiche, S., Zeghibe, K., Gharbi, S., Khelef, Y. (2017a). In-vivo study of stress oxidative and liver damage in rats exposed to acetate lead. Int. Res. J. Biological Sci. 6(9): 1-6.
61. Dibyajyoti, S. (2014). Role of Free Radicals, Oxidative Stress and Xenobiotics in Carcinogenesis by Environmental Pollutants. Avan Biomed, 3(2): 84-92, Doi: 10.1007/s12291-014-0446-0.
62. DONNA, E. (2007). European recommendations for quality assurance in screening and diagnosis of breast cancer. Piazza Amendola, 320149 1st edition.
63. Eric, R., Braverman, B.A., Carl, C. P. (1982). Essential Trace Elements and Cancer. Orthomolecular Psychiatry, 11(1): 28-41
64. Esra, B., Umit, M. S., Cansin, S., Serpil, E., Omer, K., (2012) Oxidative Stress and Antioxidant Defense. World Allergy Organ J. 5(1): 9–19. doi: 10.1097/WOX.0b013e3182439613
65. European Food Safety Authority (EFSA).(2010). Scientific Opinion on Lead in Food.
66. Fabio, H., Carolina, F. P., Luciana, B. G., Doris, R., Denise, P. C., Rodrigo, S. F. (2016). The role of oxidative stress on breast cancer development and therapy; International Society of Oncology and BioMarkers (ISOBM), DOI 10.1007/s13277-016-4873-9.
67. Fatma, V., Wanessa, C.M.A.M., Pinar, A., Daniela, V., Magesh, S., Asheesh, G., Rakkiyappan, C., Mahdi, K., Nivaldo, A. P., Rui, Y., George, P. T., Michael, R. H. (2013). Antimicrobial strategies centered around reactive oxygen species -

bactericidal antibiotics, photodynamic therapy and beyond. FEMS Microbiol Rev, 37(6), 955–989. doi:10.1111/1574-6976.12026.

68. Favier, A. (2003). Le stress oxydant: intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique. L'actualité chimiqu, 108-115.
69. Federal-Provincial-Territorial Committee on Drinking Water (CDW). (2017). Lead in Drinking Water. https://www.canada.ca/en/health-canada/programs/consultation-lead-drinking-water/document.html#a81.
70. Flora, S.J.S., Megha, M., Ashish, M. (2008). Heavy metal induced oxidative stress & it's possible reversal by chelation therapy. Indian J Med Res 128.
71. Gagan, F., Deepesh, G., Archana, T. (2012). Toxicity of lead: A review with recent updates. Interdiscip Toxicol, 5(2),47–58. Doi: 10.2478/v10102-012-0009-2.
72. Gertraud, M., Angelique, F., Johanna, E. T., Lorraine, L., Iliana, C., Jonine, F., Sarah, W. (2017). The Relation of Type 2 Diabetes and Breast Cancer Incidence in Asian, Hispanic and African American Populations-A Review.
73. Gilman, A.G., Rall, T.W., Nies, A.S., Taylor, P. eds. (1990) Goodman and Gilman's the pharmacological Basis of Therapeutics. 8th ed, NewYork, Pergamon Press.
74. Gurer-Orhan, H., Ince, E., Konyar, D., Saso, L., Suzen, S. (2017). The Role of Oxidative Stress Modulators In Breast Cancer. Current Medicinal Chemistry, DOI:10.2174/0929867324666170711114336
75. Haan, J.B., Cristiano, F., Iannello, R.C., Kola, I. (1995). Cu/Zn-superoxide dismutase and glutathione peroxidase during aging. Biochemistry and Molecular Biology International, 35(6):1281-1297
76. Hankinson S., Tamimi, R., Hunter, D. (2008). Breast cancer. Cancer epidemiology by site-specific cancers: breast cancer, in: H.O. Adami, D. Hunter, D. Trichopoulos (Eds.), Textbook of Cancer Epidemiology, 2nd ed, Oxford University Press.
77. HAS. (2011) Screening for breast cancer in France: identification of high-risk women and screening methods, www.has-sante.fr
78. Heda, D., Danielle, L. (2014). Lead Poisoning in Children. Advances in Pediatrics, 61(1), 313-33. Doi: 10.1016/j.yapd.2014.04.004.
79. Hedong, H., Wei, G., Wentao, S., Yamei, Y., Yunshuo, Z., Xiaofei, Y., Jia, H. (2017). Hypertension and breast cancer risk: a systematic review and metaanalysis; Scientific REPORTS | 7:44877 | DOI: 10.1038/srep44877

80. Hildebrand, J.S., Gapstur, S.M., Campbell, P.T., Gaudet, M.M., Patel, A.V. (2013). Recreational physical activity and leisure-time sitting in relation to postmenopausal breast cancer risk. Cancer Epidemiol Biomarkers Prev, 10.1158/1055-9965.

81. Ho, S. M., Tang, W. Y., Belmonte, 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 Res. 66:5624–32, doi:10.1158/0008-5472.CAN-06- 0516.

82. Hogg. (2007) Red meat and colon cancer: heme proteins and nitrite in the gut. Acommentary on diet-induced endogenous formation of nitroso compounds in the GI tract. Free Radic. Biol. Med. 43:1037–1039, doi:10.1016/j.freeradbiomed.2007.07.006.

83. HONG-DAR, I.W., SZE-YUAN, C., DAR-REN, C., HSIEN-WEN, K.; Differentiation of Serum Levels of Trace Elements in Normal and Malignant Breast Patients; Biological Trace Element Research, Vol. 113, 2006. Doi: 1559-0720/06/11301–0009.

84. Howell, A., Annie, S. A., Robert, B. C., Stephen, W. D., Gareth, E., Montserat, G., Andy, J. G., Timothy, J.K., John, M. S., Michelle, N. H. (2014). Risk determination and prevention of breast cancer. Breast Cancer Research, 2014, 16:446 http://breast-cancer-research.com/content/16/5/446

85. Hu, X., Juneja, S.C., Maihle, N.J., (2002). Leptinea growth factor in normal and malignant breast cells and for normal mammary gland development. J Natl Cancer Inst, 94:1704e1711.

86. Hung-Chi, Y., Mei-Ling, C., Yi-Syuan, H., Yi-Hsuan, W., Hsin-Ru, L., Hui-Ya, L., Hung-Yao, H., Daniel, Tsun-Yee, C. (2015). Glucose 6-phosphate dehydrogenase knockdown enhances IL-8 expression in HepG2 cells via oxidative stress and NF-κB signaling pathway. J Inflamm (Lond). 12: 34. doi: 10.1186/s12950-015-0078-z

87. Hursting, S. D., L. M. Lashinger, L. H. Colbert, C. J. Rogers, K.W. Wheatley, N. P. Nunez, S. Mahabir, J. C. Barrett, M. R. Forman, S. N. Perkins. (2007). Energy balance and carcinogenesis: underlying pathways and targets for intervention. Curr. Cancer Drug Targets. 7:484–491, doi:10.2174/156800907781386623.

88. Ighodaro, O.M., Akinloye, O.A. (2017). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, https://doi.org/10.1016/j.ajme.2017.09.001

89. International Agency For Research On cancer (IARC). (2006). Inorganic and organic lead compounds. Lyon, International Agency for Research on Cancer (IARC Monographs for the Evaluation of Carcinogenic Risks to Humans, Vol. 87; http://www.inchem.org/documents/iarc/vol87/volume87.pdf).

90. International Agency for Research on Cancer (IARC). (2014). Lead, https://www.cancer.org/cancer/cancer-causes/lead.html

91. International programme on chemical safety (IPC). (1994). Lead, inorganic, may. http://www.inchem.org/documents/pims/chemical/inorglea.htm

92. Iqbal, M., Younis,M., Shoukat, N., Shaikh, S., Akram, N., Abbas, R., Khawar ,A. S., Kanwal, M., Fawad ur, R. (2015). Hematological study of cancer patients with radio-chemotherapy. Science Letters, 3, (2), 75-79.

93. James, E. K., Zemin, W., Xinzhu, P., Shaoyu, Z. (2011). Oxidative stress and oxidative damage in chemical carcinogenesis. Toxicology and Applied Pharmacology, 254 (2) 86–99. Doi:10.1016/j.taap.2009.11.028.

94. Javed, S., Ali, M., Ali, F., Sanam Saiqa, A., Wajid, N. (2015). Status of oxidative stress in breast cancer patients in Pakistani population. Advancement in life sciences, 2(3),115-118.

95. Jemaia, H., Messaoudib, I., Chaouchc A., Kerkeni, A. (2007). Protective effect of zinc supplementation on blood antioxidant defense system in rats exposed to cadmium. J *Trace Elem. Med. Biol.* 21(4): 269-273.

96. Jennifer, L. K., Leslie, B. (1996). epidemiology and prevention of breast cancer. Annu. Rev. Public Health 17,47-67.

97. Jessica, M., Faupel,B., Kathleen, F.A., Jane, J.B., Heather, E., Foteini, H., Carlito, B. L., Karin, B.M., Julie, R.P., Pepper, S., Alison, M., Stuebe, C., Watson, J., Mark, E. S., Postpartum, R. (2013). Lactation, and Breast Cancer Risk: Summary of a National Cancer Institute–Sponsored Workshop. Journal of the National Cancer Institute, Volume 105, Issue 3, Pages 166-174, https://doi.org/10.1093/jnci/djs505

98. Jia-Ling, Y., Lih-Chiann, W., Chun-Yao, C., Tsung-Yun, L. (1999). Singlet Oxygen Is the Major Species Participating in the Induction of DNA Strand Breakage and 8-Hydroxydeoxyguanosine Adduct by Lead Acetate. Environmental and Molecular Mutagenesis, 33(3), 194-201. DOI: 10.1002/(SICI)1098-2280(1999)33:33.0.CO;2-O.

99. Jiao-Kun, L., Xue-Duan, L., Li, S., Wei-Min, Z., Guan-Zhou, Q., (2016). Natural plant polyphenols for alleviating oxidative damage in man: Current status and future

perspectives. Tropical Journal of Pharmaceutical Research, 15 (5), 1089-1098. doi.org/10.4314/tjpr.v15i5.27.

100. Jie, Z., Yue, Z., Ya, L., Dong-Ping, X., Sha, L., Hua-Bin, L. (2016). Spices for Prevention and Treatment of Cancers. Nutrients Review, 8, 495; doi:10.3390/nu8080495

101. John, D.O., Stuart, J., Alexandra, A., Timothy, Scott. (2013). UV Radiation and the Skin. International Journal of Molecular Sciences,*14*, 12222-12248. Doi:10.3390/ijms140612222.

102. Johnson, K.C. (2005). Accumulating evidence on passive and active smoking and breast cancer risk. Int J Cancer 117: 619-628.

103. Joint FAO/WHO Expert Committee on Food Aditives (JECFA). (2011). Evaluation of certain food additives and contaminants. http://apps.who.int/iris/bitstream/handle/10665/44515/WHO_TRS_960_eng.

104. Josko, O., Natasa, S. (2011). Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance. Journal of Clinical Toxicology, DOI: 10.4172/2161-0495.S3-001.

105. Kahlin, C.O., Guri, G., Corey, N. (2013). DNA-Damaging Agents in Cancer Chemotherapy: Serendipity and Chemical Biology. Chemistry & Biology Volume 20, Issue 5, Pages 648-659.

106. Karihtala, P., Winqvist, R., Syvaoja, J.E., Kinnula, V.L., Soini Y. (2006). Increasing oxidative damage and loss of mismatch repair enzymes during breast carcinogenesis. Eur J Cancer 24, 2653-26

107. Key, T.J., Appleby, P.H. , Reeves, G.K. , Roddam, A., Dorgan, J.F., Longcope, C. (2003). Endogenous hormones and breast cancer collaborative group body mass index, serum sex hormones, and breast cancer risk in postmenopausal women, J. Natl. Cancer Inst. 96 (16) 1218–1226.

108. Key, T.J., Pike, M.C. (1988). The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. Eur J Cancer Clin Oncol , 24: 29-43.

109. KEY, T.J., VERKASALO, P.K., BANKS, E. (2001). Epidemiology for breast cancer. Lancet Oncology , 133-140.

110. Key, T.M., Verkasalo, P.K., Banks, E. (2001). Epidemiology of breast cancer. The lancet oncology, 2.

111. Khan, S., Khoso, S. A., Memon, S., Adeel, A., Nabi, G., (2017). Study of some Hematological parameters as Biomarker for breast Cancer population of Sindh. Sindh University Research Journal (Science Series) Vol. 49 (1) 23-28 (2017).

112. Kiecolt-Glaser, J.K., Glaser, R. (1999). Psychoneuroimmunology and Cancer: Fact or Fiction?. European Journal of Cancer, 35(11), 1603-1607.

113. Kimberly, S. P., Youngjee, C., Rosalyn, W. S., Heather, F. S. (2017). Screening for breast cancer, Seminars in Oncology 44 60–72, http://dx.doi.org/10.1053/j.seminoncol.2017.02.004

114. Kirszenbaum, L. (2006). Histology and cell biology. An introduction to pathological anatomy. university boeck. P617.

115. Klaunig, J.E., Kamendulis, M.L., Hocevar, B.A. (2010) Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol 38:96–109.

116. Kuhl, C., Weigel, S., Schrading, S., (2010). Prospective multicenter cohort study to refine management recommendations for women at elevated familial risk of breast cancer: the EVA trial. J Clin Oncol , 28(9):1450–7.

117. Kyu, H.H., Bachman, V.F., Alexander, L.T., Mumford, J.E., Afshin, A.(2016). Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose-response meta-analysis for the Global Burden of Disease Study. BMJ, 354, 3857.

118. Lapayowker, M.S., Revesz, G. (1980). Thermography and ultrasound in detection and diagnosis of breast cancer. Cancer; 46(4 Suppl):933–8.

119. Laura, M. P.,Lothar R, Hajo, H.(2010).The Essential Toxin: Impact of Zinc on Human Health. Int. J. Environ. Res. Public Health, 7, 1342-1365. Doi:10.3390/ijerph7041342 .

120. Lenaz, G. (2012). Mitochondria and reactive oxygen species. Which role in physiology and pathology? Adv Exp Med Biol, 942, 93-136.

121. Leonard, S.S., Harris, G., Shi, X. (2004). Metal-induced oxidative stress and signal transduction. Free Radic Biol Me, 37(12), 1921–1942. doi:10.1016/j.freeradbiomed.2004.09.010.

122. Liang, X., Dempski, R.E., Burdette, S.C. (2016). Zn(2+) at a cellular crossroads. Curr Opin Chem Biol. 31:120-5.

123. Liao, S., Li, J., Wei, W. (2011). Association between diabetes mellitus and breast cancer risk: a meta-analysis of the literature. Asian Pac J. Cancer Prev; 12:1061e1065.

124. Lien, A. P., Hua, H., Chuong, P. (2008). Free Radicals, Antioxidants in Disease and Health. *Int J Biomed Sci,* 4 (2), 89-96.

125. Ligt, K.M., Spronk, P., Van, B., Vrancken, P., Siesling, S., Smorenburg, C.H. (2017). on behalf of the Nabon Breast Cancer Audit group ; Patients' experiences with decisions on timing of chemotherapy for breast cancer; The Netherlands, https://doi.org/10.1016/j.breast.2017.10.016

126. LITTLE, M.P., MUIRHEAD, C.R., HYLOCK, R.G., THOMAS. J.M. (1999) Relative risks of radiation-associated cancer: Comparison of second cancer in therapeutically irradiated populations with the Japanese atomic bomb survivors. Radiat. Environ. Biophys, 38: 267-83.

127. Liuen, Y., Di, P. (2012). Effects of second hand smoke on airway secretion and mucociliary clearance. Front Physiol. 28(3), 342. Doi: 10.3389/fphys.2012.00342.

128. Loghman-Adham, M. (1997). Renal Effects of Environmental and Occupational Lead Exposure. Environmental Health Perspectives,105(9),928-939.

129. Lukaszewicz-Hussain, J., Moniuszko-Jakoniuk. (2004). Liver Catalase, Glutathione Peroxidase and Reductase Activity, Reduced Glutathione and Hydrogen Peroxide Levels in Acute Intoxication with Chlorfenvinphos, an Organophosphate Insecticide; Polish Journal of Environmental Studies, Vol. 13, No. 3, 303-309.

130. Lyn, P., (2006). Lead toxicity, a review of the literature. Part I: Exposure, evaluation, and treatment. Alternative Medicine Review, 11(1), 2–22.

131. Maarten, C. B. (2005). The Role of Estrogens in Prostate Carcinogenesis: A Rationale for Chemoprevention. Rev Urol. 7(Suppl 3): S4–S10.

132. Macciò, A., Madeddu, C. (2011). Obesity, Inflammation, and Postmenopausal Breast Cancer: Therapeutic Implications. The Scientific World Journal.11:2020-2036. doi:10.1100/2011/806787.

133. Manuel, A., Nuria, V., Berta, S., Julio, L., Alejandro, T., Mónica, V., África, G. (2011). Assessment of the Evolution of Cancer Treatment Therapies. Cancers (Basel),3(3): 3279–3330. doi: 10.3390/cancers3033279

134. Maria, J.S., Marcela, B. Z., Alexis, E. L., Ricardo ,A. C., Ricardo ,W., Jose,' R. B. (2002). The Role of Zinc in the Growth and Development of Children. Nutrition, 18(6),510 –519.

135. McCormick, N.H, Hennigar, S.R, Kiselyov, K., Kelleher, S.L. (2014). The biology of zinc transport in mammary epithelial cells: implications for mammary

gland development, lactation, and involution. J Mammary Gland Biol Neoplasia 19: 59–71.

136. McPherson, M., Steel, C.M., Dixon, J.M. (2000). *ABC of Breast Diseases,* Breast cancer—epidemiology, risk factors, and genetics. BMJ, 321(7261), 624-8.

137. Mei-Ling, Y., Tso-Ying, L. (2015). A Prospective Study of the Relationship between Psychological Factors and Breast Cancer..Asia Pac J Oncol Nurs ,3, 170-5. DOI: 10.4103/2347-5625.170223.

138. Melvin R., Suersh C. (2002). Islet redox stress: the manifold toxities of Insulin Resistance, Metabolic sundrome and amylin Derived Islet Amyloid in type 2 diabetes Mellitus;Jop. J Pancreas.3 (4):86-108.

139. Michael, B. R., George, W. W., Joel, D. K. (1976). Kinetic Analysis of Lead Metabolism in Healthy Humans. The Journal of Clinical Investigation, 58(2), 260-270. doi: 10.1172/JCI108467 .

140. Michael, H. A., Susan, K. L., Steven ,W. C., Firdaus, S. D., Sandra, E. S., Paige ,G. M., Michael, S., Anil ,K. S. (2006). The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. Send to Nat Rev Cancer. 6 (3),240-8. DOI: 10.1038/nrc1820.

141. Michels, K.B, Solomon, C.G, Hu, F.B., Rosner, B.A., Hankinson, S.E., Colditz, G.A. (2003). Type 2 diabetes and subsequent incidence of breast cancer in the nurses' health study. Diabetes Care. 26:1752–1758. [PubMed: 12766105]

142. Miller, S.A., Dykes, D.D., Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16 (3):1215.

143. Mina, C. N., Mina, J. B. (2005). Modeling dynamic reciprocity: Engineering three-dimensional culture models of breast architecture, function, and neoplastic transformation. Seminars in Cancer Biology, 15 342–352, doi:10.1016/j.semcancer.2005.05.001

144. Minna, S., Liisa, P., Lauri, S., Raija, N., Paivi, R. (2017). Markku Koskenvuo, Sakari Suominen, Social support and breast cancer: A comparatory study of breast cancer survivors, women with mental depression, women with hypertension and healthy female controls; Finland 2017 ; The Breast 35 85e90; http://dx.doi.org/10.1016/j.breast.2017.06.017

145. Mladenov, M., Gokik, N., HADZI-Petrushev, I., Gjorgoski, N. (2015). The Relationship Between Antioxidant Enzymes and Lipid Peroxidation in Senescent Rat Erythrocytes; PHYSIOLOGICAL RESEARCH • ISSN 0862-8408.

146. Moinfar, F. (2007). Essentials of diagnostic breast pathology. Pratical approach. Springer-verlag Berlin Hiedelber, 496p.

147. Monterio, HP., Bechara, EJH., Abdalla, DSP.(1990). Free radicals involvement in neurological porphyrias and lead poisoning. Mol Cell Biochem, 103(1):73-83.

148. Naoki, N.(2014). Effect of Psychosocial Factors on Cancer Risk and Survival. J Epidemiol,24(1),1-6D. doi:10.2188/jea.JE20130124.

149. Nareika, A., Im, Y. B., Game, B. A., Slate, E. H., Sanders, J. J., London, S. D., Lopes-Virella, M. F., Huang, Y. (2008). High glucose enhances lipopolysaccharide-stimulated CD14 expression in U937 mononuclear cells by increasing nuclear factor kappaB and AP-1 activities. J. Endocrinol. 196:45–55, doi:10. 1677/JOE-07-0145.

150. National Cancer Institute Services (NCIS). (2007). Breast Cancer, 1-800-4-CANCER (1-800-422-6237).

151. Neukam, F.W., Stelzle, F. (2010). Laser Tumor Treatment in Oral and Maxillofacial Surgery. Physics Procedia 5 91–100

152. Nicola, T., Roberta, R., Mariapaola, N., Barbara, M., Anna, L., Maria, A. P., Umberto, M.M., Cinzia, D. (2013). Role of Glutathione in Cancer Progression and Chemoresistance. Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity, Article ID 972913, 10. Doi.org/10.1155/2013/972913.

153. Nicolas, ALSAC. (2007). Determination of heavy metals (As, Cd, Cr, Cu, Ni, Pb, Zn and Hg) in soils by ICP-MS Analysis of heavy metals (As, Cd, Cr, Cu, Ni, Pb, Zn and Hg) in soils by ICP-MS; Annals of Analytical Toxicology, vol. XIX, No. 1.

154. Niklason, L.T., Christian, B.T., Niklason, L.E. (1997). Digital tomosynthesis in breast imaging. Radiology 205(2):399–406.

155. Nkondjock, A. & Ghadirian, P. (2005). Risk Factors for Breast Cancer. M / S: Medicine Science, 21 (2), 175-180. doi: 10.7202 / 010550ar

156. Obrador E; Navarro J; Mompo J, Asensi M; Pellicer JA; Estrela JM.(1997). Glutathione and the rate of cellular proliferation determine tumour cell sensitivity to tumour necrosis factor in vivo. Biochem J;325: 183–9.

157. ODOVICO, L.B., Cancer prevention in the older individual. Oncologic Sciences and Medicine, http://dx.doi.org/10.1016/j.soncn.2016.05.011.

158. Oeffinger, K.C, Fontham, E.T., Etzioni, R. (2015). Breast cancer screening for women at average risk: 2015 guideline update from the American Cancer Society. JAMA 314(15):1599–614.

159. Onitilo, A.A., Engel, J.M., Glurich, I. (2012). Diabetes and cancer. II: Role of diabetes medications and influence of shared risk factors. Cancer Causes Control 23:991e1008.

160. Onuwa, O.P., Ishaq, S.E., Rufus, S. (2012). Analysis of heavy metals in human hair using atomic absorption spectrometry (AAS), American Journal of Analytical Chemistry, 3, 770-773. http://dx.doi.org/10.4236/ajac.2012.311102

161. Oyewopo, A.O., Olaniyi, S.K., Oyewopo, C.I., Jimoh, A.T.(2017). Radiofrequency electromagnetic radiation from cell phone causes defective testicular function in male Wistar rats. Andrologia,49(10). Doi: 10.1111/and.12772

162. Ozougwu, J. C. (2016). The role of reactive oxygen species and antioxidants in oxidative stress. International Journal of Research in Pharmacy and Biosciences, 3 (6), 1-8.

163. Panieri, E., Santoro, M.M. (2016). ROS homeostasis and metabolism: a dangerous liason in cancer cells, Cell Death Dis. 7 (6), e2253.

164. Paramasivam, R., Dominic, S., Chinthamony, A. R., Thangarajan, S., Velliyur K.G . (2012). Evaluation of Enzymatic and Non-Enzymatic Antioxidant Properties of *Aerva Lanata* (L) – An Invitro Study. International Journal of Pharmacy and Pharmaceutical Sciences, 4, (1), 522-526.

165. Parkin, M. (2006). The global health burden of infection-associated cancers in the year 2002. Int. J. Cancer. 118:3030–3044, doi:10.1002/ijc.21731.

166. Patra, R. C., Amiya, K. Rautray, Swarup, D. (2011). Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. Vet Med Int. 457327. doi: 10.4061/2011/457327

167. Patricia, I.O. (2012). Zinc and the modulation of redox homeostasis. Free Radic. Biol. Med. 53(9): 1748–1759.

168. Pelumi, E.O., Adebowale, O.A., Hilary, I. O. (2017). Breast cancer patients in Nigeria: Dataexploration approach; Nigeria, Data in Brief 15 47–57, 2017 http://dx.doi.org/10.1016/j.dib.2017.08.038

169. Peter, M., William, T., Mohammed, H., Vikram, P., Damir, J., Luca, C.(2010). Pathophysiological Impact of Cigarette Smoke Exposure on the

Cerebrovascular System with a Focus on the Blood-brain Barrier: Expanding the Awareness of Smoking Toxicity in an Underappreciated Area. International Journal of Environmental Research and Public Health, 7, 4111-4126; doi: 10.3390/ijerph7124111.

170. Petrakis, N.L., Ernster, V.L., King, C.M. (2006). Breast cancer, in : D. Schottenfeld, J.F. Fraumeni (Eds.), Cancer Epidemiology and Prevention, 3rd ed.

171. Pincemail, J. M., Meurisse, R., Limet, J.O., Defraigne. (1999). Espèces oxygénées activées, antioxydants et cancer Vaisseaux, Coeur, Poumons Vol 4 N°4.

172. Pisani, D. M. Parkin, N. Munoz, and J. Ferlay.(1997). Cancer and infection: estimates of the attributable fraction in 1990. Cancer Epidemiol. Biomarkers Prev. 6:387–400.

173. Pizot C, Boniol M, Mullie P, Koechlin A, Boniol M, et al. (2016). Physical activity, hormone replacement therapy and breast cancer risk: A meta-analysis of prospective studies. Eur J Cancer, 52, 138-54.

174. Ponce-Canchihuaman, J.C., Pérez-Méndez,O ., Hernandez- Munoz ,R ., Torres-Durán P.V.,Juarez-Oropeza,M.A.(2010).protecive effects of Spirulina maxima on hyperlipidemia and oxidative –stress induced by lead acetate in the liver and Kidney. Lipids in Health and Disease,9(35).

175. Posch, H., Seitz, K. (2004). Alcohol and cancer. Alcohol Alcohol. 39:155–65 doi:10.1093/alcalc/agh057.

176. Prasad, A.B., F.W. Endre, L., Handschu, W., Kukuruga, M., Kamar, G. (1996). Zinc deficiency affects cell cycle and deoxythymidine kinase gene expression in HUT-78 cells. J. Clin. Med. 128: 51-60.

177. Preetha, A., Ajaikumar, B. K., Chitra, S., Kuzhuvelil, B. H., Sheeja ,T. T., Oiki, S. L., Bokyung, S., Bharat, B. A. (2008). Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharmaceutical Research, 25(9). DOI: 10.1007/s11095-008-9661-9.

178. Public Health Agency of Canada (PHAC). (2009). What are Your Risks of Breast Cancer.

179. Public Health England (PHE). (2017). Lead Toxicological Overview, publications gateway number: 2014790, www.nationalarchives.gov.uk/doc/open-government-licence/version/3/

180. Public health England (PHE). (2017). Lead Toxicological overview. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/653725/Lead_toxicological_overview.

181. Puddu M., Tafforeau J. (2005). Opportunity to screen for breast cancer among women aged 40 to 49, State of knowledge and data available for the development of a health policy in Belgium, Scientific Institute of Public Health.

182. Putnam, R.D. (1986). Review of toxicology of inorganic lead. Am. Ind. Hyg. Assoc. J, 47(11), 700-703.

183. Rachael, T., Leon, G., Rachel, N., Lynne, R. W., Marie, C., Kami, K.W., Yurii, B.S., Arielle, B., Grazyna, B., Michelle, B. (2017). Risk factors for breast cancer in the breast cancer risk model study of Guam and Saipan ; International Journal of Cancer Epidemiology, Detection, and Prevention, Volume 50, Part B, Pages 221-233; http://dx.doi.org/10.1016/j.canep.2017.04.008.

184. Raeve HR, Thunnissen FB, Kaneko FT, Guo FH, Lewis M, Kavuru MS, Secic M, Thomassen MJ, Erzurum SC; Decreased Cu,Zn-SOD activity in asthmatic airway epithelium: correction by inhaled corticosteroid in vivo; 1997 Jan;272(1 Pt 1):L148-54; DOI: 10.1152/ajplung.1997.272.1.L148

185. Rakesh K. G., Neeta, S. (2013). Morinda citrifolia (Noni) Alters Oxidative Stress Marker and Antioxidant Activity in Cervical Cancer Cell Lines. Asian Pacific Journal of Cancer Prevention, 14 (8), 4603-4606. doi.org/10.7314/APJCP.2013.14.8.460.

186. Rakesh, K. G., Amit, K. P., Niranjan, S., Arun, K. C., Uday, K. J., Uday, C. Y., Pavan, K. G., Uttam, P. (2014). Oxidative Stress and Antioxidants in Disease and Cancer: A Review. Asian Pacific Journal of Cancer Prevention, 15(11), 4405-4409. doi.org/10.7314/APJCP.2014.15.11.4405.

187. Randy Y.C. Poon, Randy Y.C. Poon. Gene mutation and aneuploidy The Instability that Causes Cancer. Cell Cycle 3:9, 1101-1103; 2004

188. Ranneh, Y., Fadel, A. (2017).Crosstalk between reactive oxygen species and pro-inflammatory markers in developing various chronic diseases: a review. Journal of the Korean Society for Applied Biological Chemistry, 60(3), 327-338. doi.org/10.1007/s13765-017-0285-9.

189. Rao N, Chen K, Yang Q, Niu J, A proof-of-concept study of 3D-printed moldguided breast-conserving surgery in breast cancer patients, Clinical Breast Cancer (2018), doi: 10.1016/j.clbc.2018.02.005.

190. Rashmi, B., Protiti, B., Vikram, S. G., Lalit, K. (2017). The Role of ROS in COPD Progression and Therapeutic Strategies. Reactive Oxygen Species, 4(10):237–250. doi: 10.1016/j.nupar.2006.10.178.

191. Raúl Baena Ruiz, Pedro Salinas Hernández; Diet and cancer: Risk factors and epidemiological evidence; Department of Medical Oncology, Hospital La Zarzuela, Madrid, Spain; 2013. http://dx.doi.org/10.1016/j.maturitas.2013.11.010

192. Raymond W Ruddon; cancer biology; 2007; oxford, New York: oxford university press; 2007; 4 edition; p04.p487.

193. Riedl CC, Luft N, Bernhart C, et al. Triple-modality screening trial for familial breast cancer underlines the importance of magnetic resonance imaging and questions the role of mammography and ultrasound regardless of patient mutation status, age, and breast density. J Clin Oncol 2015;33(10): 1128–35.

194. Rinne, D. B., Nik, V. L. (2004). Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis, 19(3), 169-185. DOI: 10.1093/mutage/geh025.

195. Robert W Jeffery, Judy Baxter, Maureen McGuire and Jennifer Linde; Are fast food restaurants an environmental risk factor for obesity?; International Journal of Behavioral Nutrition and Physical Activity; USA; 2006.

196. Rupen Shah, Kelly Rosso, S David Nathanson ; Pathogenesis, prevention, diagnosis and treatment of breast cancer ; Published by Baishideng Publishing Group Inc 8226 Regency Drive, Pleasanton, CA 94588, USA ;2014.

197. Saddig D. J. (2016). Is Long-Term Cell Phone Use Linked to Breast Cancer? A Review of the Evidence. Advances in Breast Cancer Research, 5, 136-141. Doi.org/10.4236/abcr.2016.54016.

198. Sarawoot, P., Phanit, K. (2015).Oxidative Stress-Associated Pathology: A Review (Patologi berkaitan Tekanan Oksidatif: Suatu Kajian). Sains Malaysiana, 44 (10), 1441–1451. DOI: 10.17576/jsm-2015-4410-09.

199. Sardanelli F, Podo F, Santoro F, et al. Multicenter surveillance of women at high genetic breast cancer risk using mammography, ultrasonography, and contrast-enhanced magnetic resonance imaging (the high breast cancer risk Italian 1 study): final results. Invest Radiol 2011;46(2):94–105.

200. Saroj, S. G., Devasagayam, TPA., Tilak, J., Boloor, KK., Ketaki, S. S., Lele, RD. (2004). Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. The Journal of the Association of Physicians of India, 52, 794-804.

201. Sergio, C. S., Alberto, I. (2013). Focus on molecular events in the anterior chamber leading to glaucoma. Cell Mol Life Sci, 71(12), 2197-218. Doi: 10.1007/s00018-013-1493-z.

202. Serguei A. Castaneda, MD, Jon Strasser, MD; Updates in the Treatment of Breast Cancer with Radiotherapy; Cancer Center & Research Institute; 2017 ; http://dx.doi.org/10.1016/j.soc.2017.01.013

203. Shihong, L., Christian, S., Ronald, T. B. (1995). Chemical Instability of Protein P harmaceuticals: Mechanisms of Oxidation and Strategies for Stabilization. Biotechnology and Bioengineering, 48(5), 490-500. DOI: 10.1002/bit.260480511.

204. Shilpa, B., Rima, D. (2017). Oxidative stress: Major executioner in disease pathology, role in sperm DNA damage and preventive strategies. Frontiers in Bioscience, 9, 420-447.

205. Shiwani Patil and N. Saraswathy; ENZYMES AS TARGETS FOR CANCER THERAPY; Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, SVKM`s NMIMS, Vile Parle (W), Mumbai - 400056, Maharashtra, India.2017.

206. Shrivastava, S., Neelima, S., Akshay, K. N., Sanjay, S. C., Reetika, S., Sarvesh,K. (2017). Comparative study of hematological parameters along with effect of chemotherapy and radiotherapy in different stages of breast cancer. International Journal of Research in Medical Sciences, 5(1):311-315. DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20164569.

207. Sickles EA, Filly RA, Callen PW. Breast cancer detection with sonography and mammography: comparison using state-of-the-art equipment. AJR Am J Roentgenol 1983;140(5):843–5.

208. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66:7-30.

209. Sivasakthivel.T ., K.K.Siva Kumar Reddy. (2011). Ozone Layer Depletion and Its Effects: A Review. International Journal of Environmental Science and Development, 2 (1).

210. Smith RA, Duffy SW, Gabe R, et al. The randomized trials of breast cancer screening: what have we learned ? Radiol Clin North Am 2004; 42(5):793– 806, v.

211. Song S., H. C. Pitot, and P. F. Lambert. The human papillomavirus type 16 E6 gene alone is sufficient to induce carcinomas in transgenic animals. J. Virol. 73:5887–5893 (1999).

212. Sonia Tusell Wennier, Jia Liu, and Grant McFadden. Bugs and Drugs: Oncolytic Virotherapy in Combination with Chemotherapy. Curr Pharm Biotechnol. 2012 Jul; 13(9): 1817–1833.

213. Srogi; Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review; Institute for Chemical Processing of Coal, Zabrze, Poland; November 2007, Volume 5, Issue 4, p 169–195

214. Staessen, J. A., Wang, J., Bianchi, G. & Birkenhager, W. H. Essential hypertension. Lancet 361, 1629–1641 (2003).

215. Stipanuk B.L., Falchuk K.H. Biochemical, Physiological, Molecular Aspects of Human Nutrition. 2nd. Saunders/Elsevier; St. Louis, MO, USA: 1993. pp. 1044–1064.

216. Szabo, P. Mandrekar, S. Oak, and J. Mayerle. Effect of ethanol on inflammatory responses. Implications for pancreatitis. Pancreatology.7:115–23 (2007) doi:10.1159/000104236.

217. T. Ishimoto, O. Nagano, T. Yae, M. Tamada, T. Motohara, H. Oshima, et al., CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(-) and thereby promotes tumor growth, Cancer Cell, Data en Brrief, 2011, Doi: 10.1016/j.ccr.2011.01.038.

218. Tabar L, Yen AM, Wu WY, et al. Insights from the breast cancer screening trials: how screening affects the natural history of breast cancer and implications for evaluating service screening programs. Breast J 2015; 21(1):13–20.

219. Tahari Z., A. Medjdoub, T. Sahraoui, F.Z. Tahari, R. Soulimani, A. Bousahba, F.Z. El Kebir ; Analyse de l'état du stress oxydatif dans le cancer du sein à l'Ouest algérien via les tests FORD et FORT et corrélation avec le grade histopronostique SBR ; J. Afr. Cancer (2013) 5:16-21 DOI: 10.1007/s12558-012-0236-7

220. Taheripanah R, Balash F, Anbiaee R, Mahmoodi M, Akbari Sene A,Breast Cancer and Ovulation Induction Treatments, Clinical Breast Cancer (2018), doi: 10.1016/j.clbc.2018.03.003.

221. Tang H., Y. C. Chiu, T. W. Tan, R. S. Yang, and W. M. Fu. Adiponectin enhances IL-6 production in human synovial fibroblast via an AdipoR1 receptor, AMPK, p38, and NFkappa B pathway. J. Immunol. 179:5483–5492 (2007).

222. Teresa Norata Chiara Scocciantib Marie-Christine Boutron-Ruaultc Annie Andersond Franco Berrinoe Michele Cecchinif Carolina Espinab Tim Keyg Michael Leitzmannh Hilary Powersi Martin Wisemanj Isabelle Romieub. European Code

against Cancer 4th Edition: Diet and cancer. Cancer Epidemiology. 2015 . 39(1): S56-S66.

223. The American Cancer Society medical and editorial content team (2014). Evolution of Cancer Treatments: Hormone Therapy

224. The Cational Cancer Institute, Understanding breast cancer, 52, avenue André Morizet 92 513 Boulogne Billancourt CEDEX, ISBN: 978-2-913 495-30-2; Validation: January 2007, 1st reissue November 2007

225. Thorburne, S.K., Juurlink, B.H. (1996). Low glutathione and high iron govern the susceptibility of oligodendroglial precursors to oxidative stress. J Neurochem, 67(3), 1014-22.

226. Tohru, Y., Hironobu, Y., Masayuki, Y., Yusuke, W., Yoshinari, N., Hideo, N., Osamu, I.(2012). Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function and mitochondrial content under control of the cell cycle checkpoint. Free Radical Biology and Medicine, 53(2), 260-270. doi: 10.1016/j.freeradbiomed.2012.04.033.

227. Tomasz Wilmanski, Xuanzhu Zhou, Wei Zheng, Aparna Shinde, Shawn S. Donkin, Michael Wendt, John R. Burgess, Dorothy Teegarden, Inhibition of pyruvate carboxylase by 1a,25-dihydroxyvitamin D promotes oxidative stress in early breast cancer progression, Cancer Letters (2017), https://doi.org/10.1016/j.canlet.2017.09.045

228. Tulin D. Cil, MD, MEd, FRCSC, David McCready, MD, MSc, FRCSC ; Modern Approaches to the Surgical Management of Malignant Breast Disease The Role of Breast Conservation, Complete Mastectomy, Skin- and Nipple-Sparing Mastectomy ; Clin Plastic Surg 45 (2018) 1–11 ; http://dx.doi.org/10.1016/j.cps.2017.07.002

229. Tulinius, H. Sigvaldason and G. Olafsdottir; Left and Right Sided Breast Cancer; The Icelandic Cancer Registry and the Department of Preventive Medicine, University of Iceland, Reykjavfk, Iceland,1990; DOI: 0344-0338/9010186-0092$3.5010

230. Ulla, P., Peeter, K. (2015). Oxidative stress in the pathogenesis and prognosis of ovarian cancer. acta universitatis ouluensis d medica 1323.

231. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 2002; 2: 171-82.

232. Viduranga, W., Lee, Y. H. (2013). A Comparative Study on the Antioxidant Activity of Commonly Used South Asian Herbs. J Tradit Complement Med, 3(4), 263–267. Doi: 10.4103/2225-4110.119725.

233. Vikram, J., Koji, U., Vasanthy, N. (2012). Pathophysiology of Lipoprotein Oxidation. doi.org/10.5772/506.

234. Volodymyr, I. L. (2014). Classification of Oxidative Stress Based On Its Intensity. Excli Journal, 13, 922-937 – ISSN 1611-2156.

235. Wang X.J., Z. Sun, N.F. Villeneuve, S. Zhang, F. Zhao, Y. Li, et al., Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2, Carcinogenesis 29 (6) (2008) 1235e1243.

236. WECKBEKER G., CORY J.G., 1988- Ribonucleotide reductase activity and growth of Glutathoine-depleted mous leikemia L1210 cells in vitro. Cancer letters. vol. 40: 257-264.

237. Weigert J, Steenbergen S. The Connecticut experiment: the role of ultrasound in the screening of women with dense breasts. Breast J 2012;18(6): 517–22.

238. Weyer PJ1, Cerhan JR, Kross BC, Hallberg GR, Kantamneni J, Breuer G, Jones MP, Zheng W, Lynch CF. ; Municipal drinking water nitrate level and cancer risk in older women: the Iowa Women's Health Study; Center for Health Effects of Environmental Contamination, University of Iowa, USA. ;2001 May;12(3):327-38.

239. Willett W. C.. Diet and cancer. Oncologist. 5:393–404 (2000) doi:10.1634/theoncologist.5-5-393.

240. Williamson J.R., Kilo C. (1989). Glucotoxicity and diabetic vascular disease. Medical dialogue on diabetes. 8: 8-10.

241. World Cancer Research Fund/American Institute for Cancer Research. Continuous update project report: food, nutrition, physical activity and the prevention of breast cancer, 2010.

242. World Health Organization (WHC), Cancer, 2015, http://www.who.int/fr/news-room/fact-sheets/detail/cancer.

243. World Health Organization (WHO) (2010). Childhood lead poisoning.

244. World Health Organization (WHO), Infant and Young Child Feeding: Model Chapter for Textbooks for Medical Students and Allied Health Professionals, 2009

245. World Health Organization (WHO), Lead poisoning and health, 2018. http://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

246. World Health Organization (WHO), Obesity and Overweight Fact Sheet,

2012 (May, http://www.who.int/mediacentre/factsheets/fs311/en/).

247. World Health Organization (WHO), Preventing disease through healthy environments exposure to lead: a major public health concern, 2010.

248. World Health Organization (WHO). (2001). Air quality guidelines for Europe. w.euro.who.int/en/health-topics/environment-and-health/air-quality/publications/pre2009/who-air-quality-guidelines-for-europe,-2nd-edition,-2000-cd-rom-version.

249. World Health Organization (WHO). (2010). Exposure To Lead: A Major Public Health Concern.

250. World Health Organization (WHO). (2011). Evaluation of certain food additives and contaminants. http://apps.who.int/iris/bitstream/handle/10665/44515/WHO_TRS_960_eng.pdf;jsessionid=8045F5D3DC9BDB7D583DE93F7FE6101B?sequence=1

251. Wu Xiujuan, Jing Tang, Mingjun Xie; Serum and hair zinc levels in breast cancer: a meta-analysis; 2015, Scientific REPORTS 5:12249. DOi: 10.1038/srep12249

252. Xiao Ding, Meng Jiang, Haiyan Jing, Wei Sheng, Xingwen Wang, Junqing Han, Luhua Wang; Analysis of serum levels of 15 trace elements in breast cancer patients in Shandong, China; Environ Sci Pollut Res; 2014;DOI 10.1007/s11356-014-3970-9

253. Xiao, L. (2012). Ultraviolet a Radiation, Reactive Oxygen Species and NF-kappa B. Pharmaceut Reg Affairs, 1(107). doi:10.4172/2167-7689.1000e107.

254. Xue F, Michels KB. Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. Am J Clin Nutr 2007;86:s823es835.

255. YAGI K., Simple Fluorometric Assay for lipoperoxyde in blood plasma. Biochemical. Medecine. vol. (15) : 212-216. Dosage of reduced glutathione (GSH), 1976.

256. Yiu Wai-ki, Maria T Basco, John E Aruny, Stephen WK Cheng, and Bauer E Sumpio. Cryosurgery: A review. Int J Angiol. 2007 Spring; 16(1): 1–6.

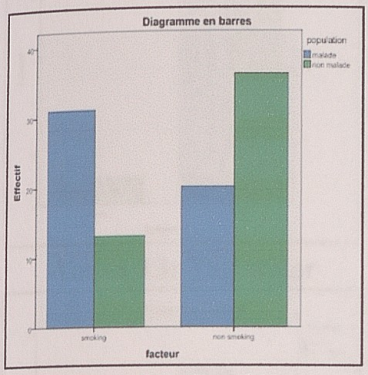
257. Zaki H.M, Garba-Bouda O, Garba S.M, Nouhou H. (2013). Epidemiological and pathological profile of breast cancer in Niger. Afr. Cancer, DOI: 10.1007/s12558-013-0274-9.

258. Zareh rahime, Wrya Parwaie, Bagher Farhood, Mahdieh Afkhami Ardekani, Hossein Safari, Evaluating the frequency of breast cancer risk factors in women referred to mammography center for breast cancer screening: a report from south part of Iran, 2018; Journal of Cancer Policy https://doi.org/10.1016/j.jcpo.2018.04.004

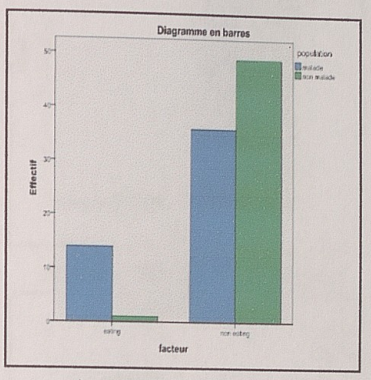
259. Zhiyou, C., Liang-Jun Y. (2013). Protein Oxidative Modifications: Beneficial Roles in Disease and Health. J Biochem Pharmacol Res, 1(1), 15–26.

Annex

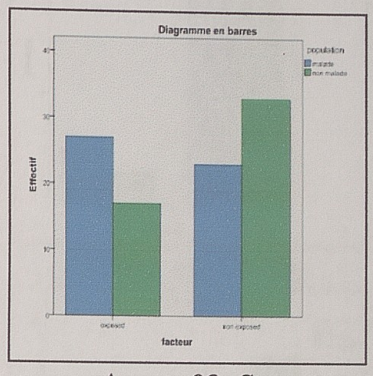
Percentage of present or absent of risk factors in breast cancer patients and controls



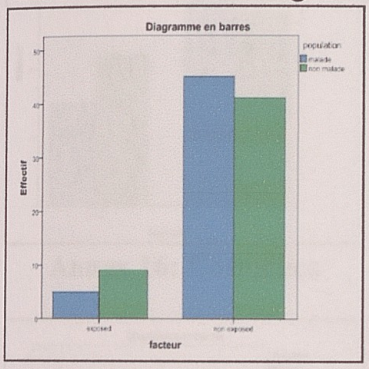
Annex01: Smoking



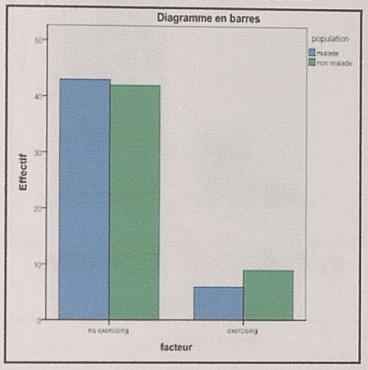
Annex02: Fastfoods



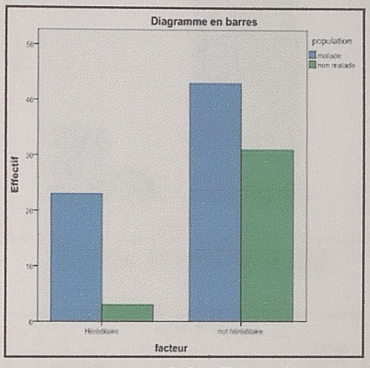
Annex 03: Sun



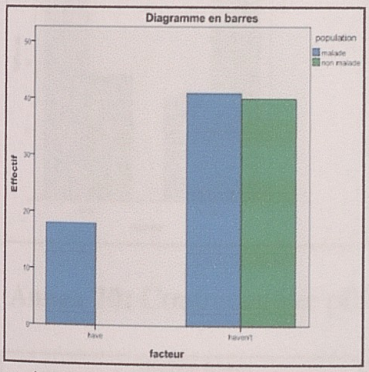
Annex 04: Chemicals substances



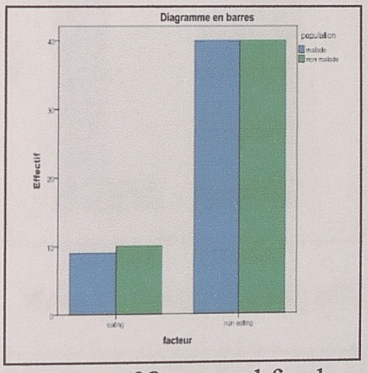
Annex 05: Sport



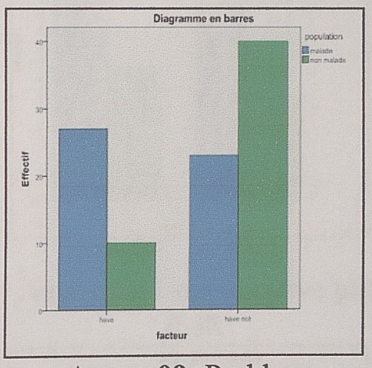
Annex 06: Genetic



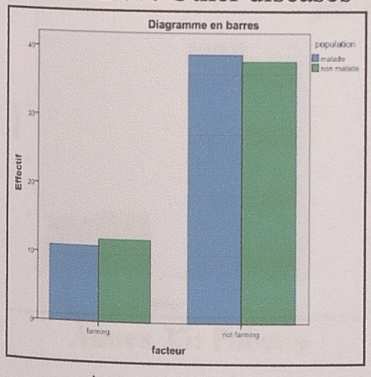
Annex 07: Other diseases



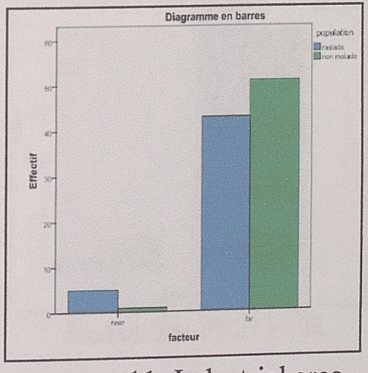
Annex 08: canned foods



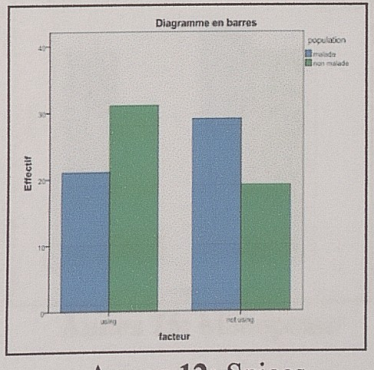
Annex 09: Problems



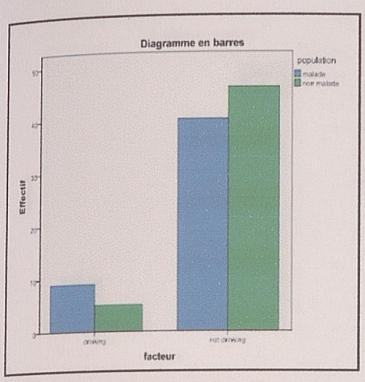
Annex 10: Farm



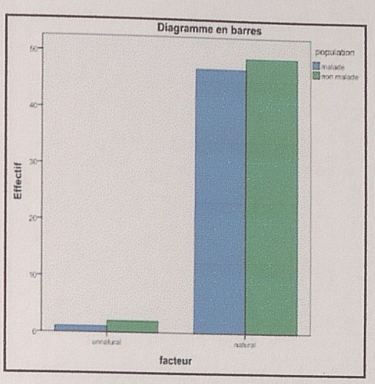
Annex 11: Industrial area



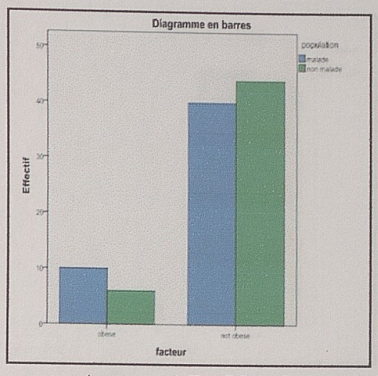
Annex 12: Spices



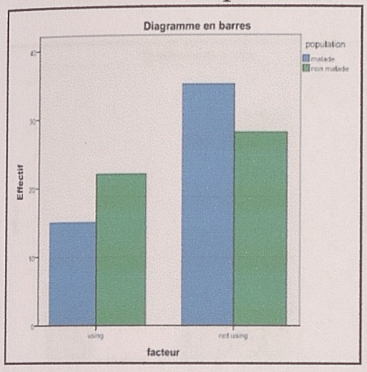
Annex 13: Tap water



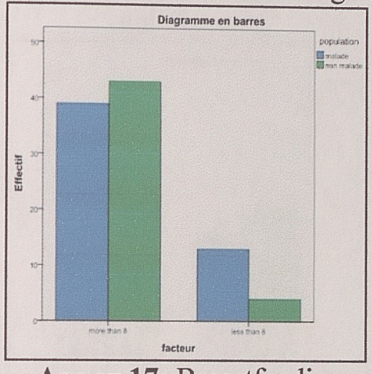
Annex 14: Breastfeeding



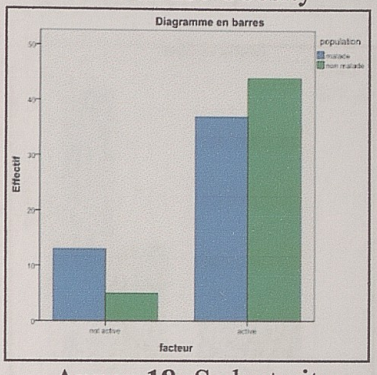
Annex 15: Obesity



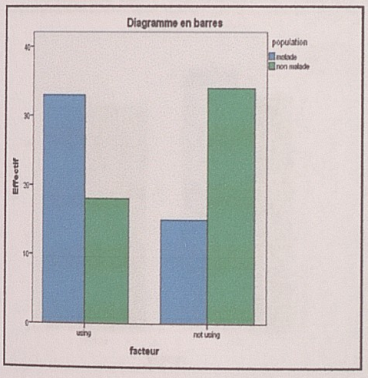
Annex 16: Cosmetics



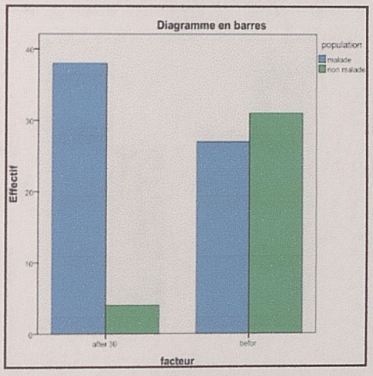
Annex 17: Breastfeeding duration



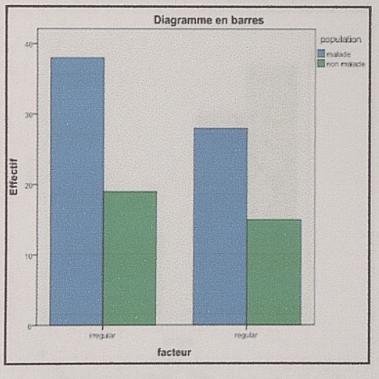
Annex 18: Sedentarity



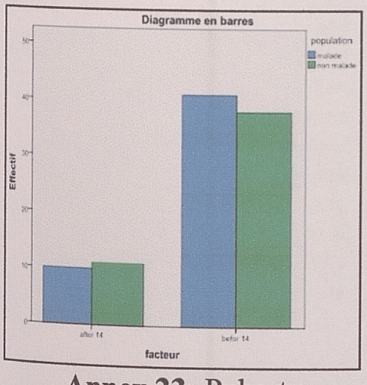
Annex 20: Contraceptive pill



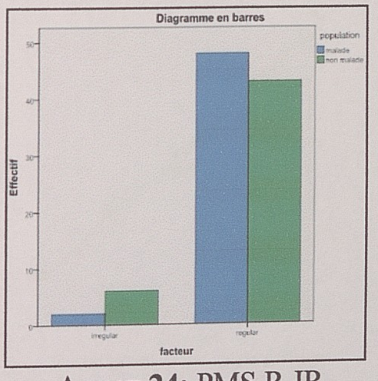
Annex 21: Age of using pill



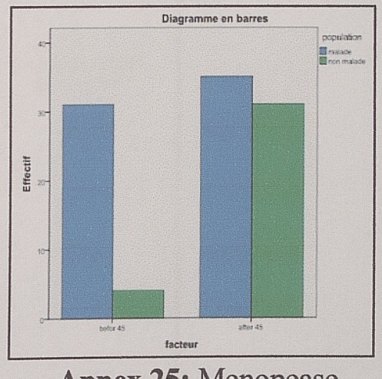
Annex 22: Contraceptive pill R-IR



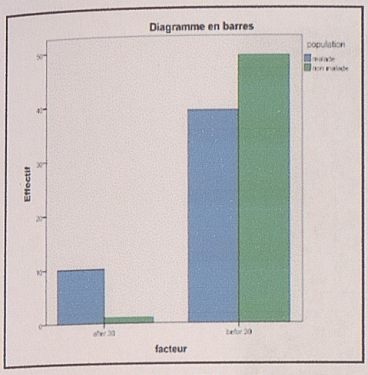
Annex 23: Puberty



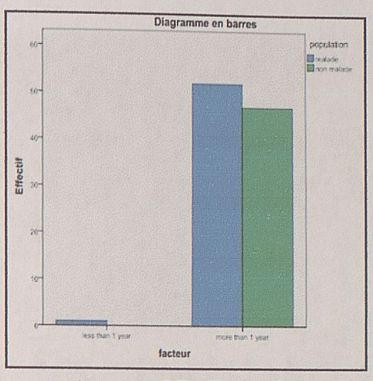
Annex 24: PMS R-IR



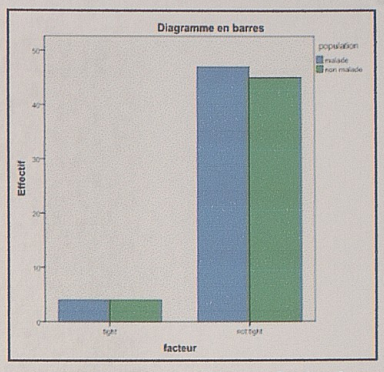
Annex 25: Menopease



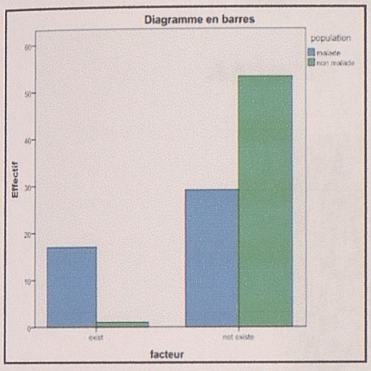
Annex 26: First born



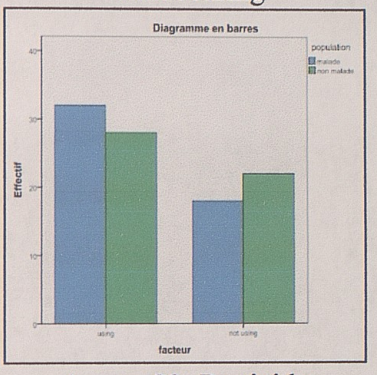
Annex 27: Duration childbearing



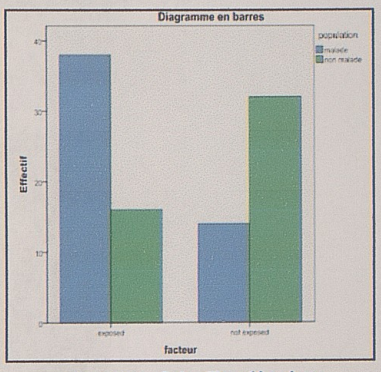
Annex 28: Quality of clothes



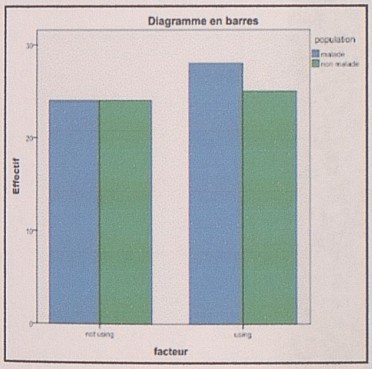
Annex 29: Phone in bras



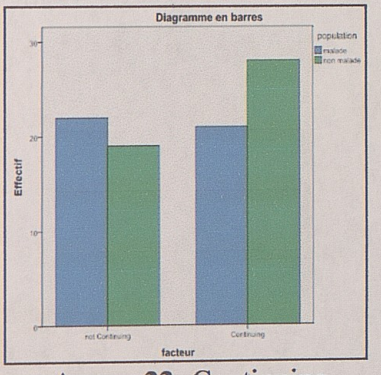
Annex 30: Pesticides



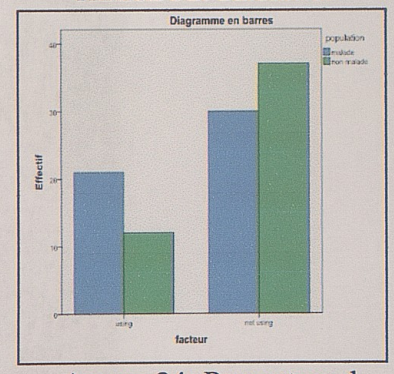
Annex 31: Radiation



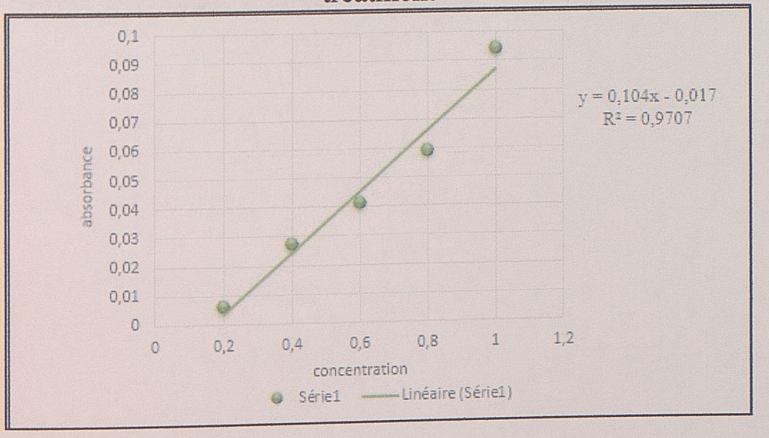
Annex 32: Herbs



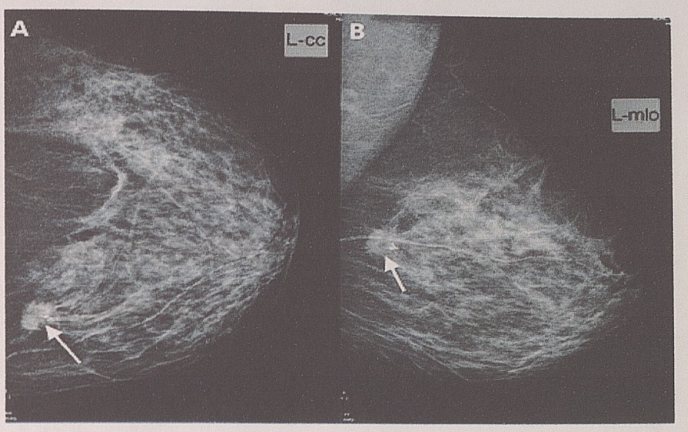
Annex 33: Continuing treatment



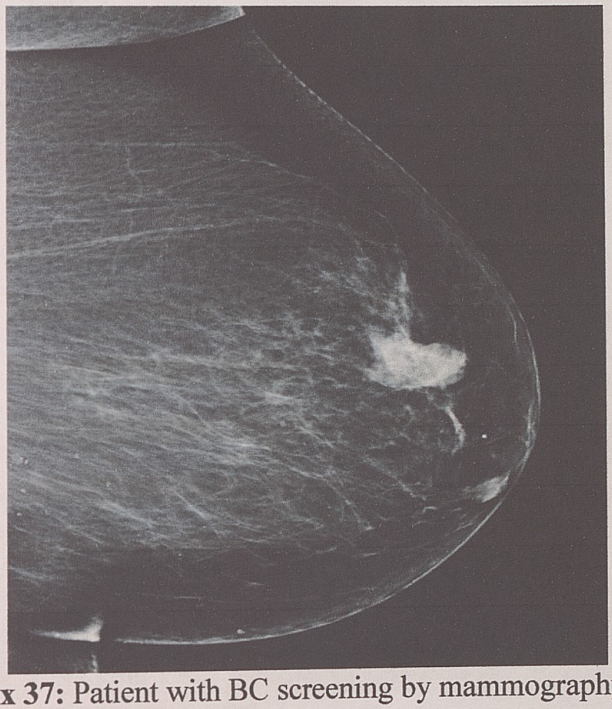
Annex 34: Paracetamol



Annex 35: Calibration curve of BSA



Annex 36 : Patient with BC screening by mammography (1)



Annex 37: Patient with BC screening by mammography (2)