FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TURKEY

DETERMINATION OF SERUM TOTAL ANTIOXIDANTS AND MELATONIN HORMONE LEVELS ON PATIENTS WITH BREAST CANCER

Amjad Mahmood QADIR

Master's Thesis

DEPARTMENT OF CHEMISTRY

Division of Biochemistry

FEBRUARY 2021

FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TURKEY

Department of Chemistry

Master's Thesis

DETERMINATION OF SERUM TOTAL ANTIOXIDANTS AND MELATONIN HORMONE LEVELS ON PATIENTS WITH BREAST CANCER

Author
Amjad Mahmood QADIR

Supervisor
Prof. Dr. Ayşegül YAZICI

FEBRUARY 2021
ELAZIG

FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TURKEY

Department of Chemistry

Master's Thesis

Title:	Determination Of Serum Total Antioxidants And Melatonin Hormone Levels On Patients With Breast Cancer
Author:	Amjad Mahmood QADIR
Submission Date:	18 January 2021
Defense Date:	2 February 2021

THESIS APPROVAL

This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Fırat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

Signature

Supervisor:	Prof. Dr. Ayşegül YAZICI Firat University, Faculty of Science	Approved
Member:	Prof. Dr. Mustafa KARATEPE Firat University, Faculty of Science	Approved
Member:	Dr. Öğr. Üyesi.Hakan ŞAHAL Munzur University.Tunceli MYO	Approved

This thesis was approved by the Administrative Board of the Graduate School on
..... / ..... / 20 .....

Signature

Doç. Dr. Kürşat Esat ALYAMAÇ
Director of the Graduate School

DECLARATION

I hereby declare that I wrote this Master's Thesis titled “Determination Of Serum Total Antioxidants And Melatonin Hormone Levels On Patients With Breast Cancer” in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

2 February 2021

Amjad Mahmood QADIR

PREFACE

Breast cancer is the most common type of cancer in the world. It is an oncological disease that causes death in humans. In this disease, the cells of the breast divide without control. Melatonin is a hormone that is secreted by the Pineal gland that causes decreasing breast cancer risk factors. On the other hand, Antioxidants are dietary products that are required to protect DNA and cells from oxidative damage. Total Antioxidant Capacity is defined as a factor that causes to increase in the activity of all the antioxidants in humans.

First and foremost thanks to Allah that gave me a healthy and chance for completing this thesis. Also, I would like to express my special thanks of gratitude to both my supervisor Prof. Dr. Ayşegül YAZICI and Prof. Dr. Mustafa KARATEPE who gave me the golden opportunity to do this wonderful project and helped me to write this research and I came to know so many new things. Special thanks to (Faculty of veterinary medicine) who helped me to read my samples by using their ELISA device.

Secondly, I would also like to thank my parents, and friends who helped and supported me in finalizing this project especially Mr. Edres Muhamad and Dr. Rebaz Anwar that supported, helped, and took a lot of time with me for writing and studying about this project.

This work has been supported by the Management Unit of Scientific Research Projects of Firat University (FUBAP) (Project Name: determination of serum total antioxidants and melatonin hormone levels on patients with breast cancer) and (Project Number: FF.19:35).

Amjad Mahmood QADIR
ELAZIG 02-02- 2021

TABLE OF CONTENTS

ABSTRACT

Determination Of Serum Total Antioxidants And Melatonin Hormone Levels On Patients With Breast Cancer

Amjad Mahmood QADIR

Master's Thesis

FIRAT UNIVERSITY
Graduate School of Natural and Applied Sciences

Department of Chemistry

February 2021, Page: xii + 63

Breast cancer is the most common type of cancer in women worldwide. Cancer of the breast is a heterogeneous disease related to different biological characteristics and clinical actions. The cells of the breast divide without control. Melatonin is a hormone that is synthesized and released by the pineal gland in the brain and responds to the sleep/wake cycle in the body. Melatonin acts as a free radical scavenger and antioxidant. Antioxidants are substances that protect cells against free radicals and cause delay or prevent oxidation. The aim of this study is the determination of serum total antioxidants and melatonin levels on patients with breast cancer and compare them to the healthy group. In this thesis, enzyme-linked immunosorbent assay (ELISA) was used to determine serum total antioxidant levels and high-performance liquid chromatography (HPLC) was used to measure the serum levels of melatonin in humans. According to our results, serum total antioxidants and melatonin levels in both the patient and control groups reached a maximum at 2:00 in the night and declined to a minimum at 9:00 in the morning but it was significantly high in the control group compared to the patient group.

Keywords: Breast cancer, Melatonin, Total antioxidants, Free radicals, Oxidative stress, HPLC, ELISA

ÖZET

Meme Kanserli Hastalarda Serum Total Antioksidanlar ve Melatonin Hormon Düzeylerinin Belirlenmesi

Amjad Mahmood QADIR

Yüksek Lisans Tezi

FIRAT ÜNİVERSİTESİ
Fen Bilimleri Enstitüsü

Kimya Anabilim Dalı

Şubat 2021, Sayfa: xii + 63

Meme kanseri, dünya çapında kadınlarda en sık görülen kanser türüdür. Meme kanseri, farklı biyolojik özellikler ve klinik etkilerle değişiklik gösteren bir hastalıktır. Meme hücreleri kontrolsüz olarak bölünür. Melatonin beyindeki epifiz bezi tarafından sentezlenip salgılanan ve vücuttaki uyku / uyanma döngüsünden etkilenen bir hormondur. Melatonin, serbest radikal önleyici ve antioksidan görevi görür. Antioksidanlar, hücreleri serbest radikallere karşı koruyan ve oksidasyonu geciktiren veya engelleyen maddelerdir. Bu çalışmanın amacı; meme kanserli hastalarda serum total antioksidan ve melatonin düzeylerinin tespit edilmesi ve sağlıklı kontrol grubuyla karşılaştırılmasıdır. Bu tezde, insanlarda serum toplam antioksidan seviyelerini belirlemek için enzime bağlı immünosorbent testi (ELISA) ve melatonin serum seviyelerini ölçmek için yüksek performanslı sıvı kromatografisi (HPLC) kullanılmıştır. Sonuçlarımıza göre, hem hasta hem de kontrol grubundaki serum toplam antioksidan ve melatonin düzeyleri gece saat 2: 00 de maksimuma ulaştı ve sabah saat 9: 00'da minimuma düştü ancak hasta grubuna göre kontrol grubunda oldukça yüksekti.

Anahtar Kelimeler: Meme Kanseri, Melatonin, Toplam Antioxidanlar, Serbest Radikaller, Oksidatif Stres, HPLC, ELISA

LIST OF FIGURES

Page

LIST OF TABLES

LIST OF APPENDICES

SYMBOLS AND ABBREVIATIONS

Abbreviations

CA	: Cancer
BC	: Breast cancer
MLT	: Melatonin
SCN	: Suprachiasmatic nucleus
FRs	: Free radicals
AOs	: Antioxidants
GPx	: Glutathione peroxidase
SOD	: Superoxide dismutase
CAT	: Catalase
NO	: Nitric oxide
GSH	: Glutathione
GST	: Glutathione s-transferase
Es	: Estrogens
CRs	: Circadian rhythms
OH•	: Hydroxyl radical
ROS	: Reactive oxygen species
RNS	: Reactive nitrogen species
OS	: Oxidative Stress
GH	: Growth Hormone
TAC	: Total Antioxidant Capacity

1. INTRODUCTION

Cancer (CA) is a leading cause of death is accountable for 13% of all deaths worldwide and is the second leading cause in the United States. It is the prospective five-year existence rate through all cancers that has risen around 65% nowadays. These achievements have been succeeded largely by powerful mediations including radiation therapy, chemotherapy, and surgery. There are many different types of cancers such as breast, lung, melanoma, colon, ovarian, prostate, kidney, brain, bone, gastrointestinal, urinary tract, lymphomas, leukemia, thyroid, retinoblastoma, sarcoma, central nervous system, rectal and pancreatic. Cancers are widely can be seen in some countries, including Argentina, Western Europe, Northern America, Norway, and Denmark. Many significant factors cause increasing breast cancer but the precise risk factors behind this finding are not well known. As a contributing cause of breast cancer, exposure to light at night, including disruption of the circadian rhythm, likely mediated through melatonin synthesis and clock genes, has been recommended [1, 2].

Breast cancer (BC) is the most common type of cancer in women worldwide. It's accounting for one-quarter of all cancers in women. Many factors cause to increase in this kind of disease but the strongest risk factors are age, sex, and quality of life. There is evidence that age is the most significant factor in endogenous melatonin [3]. Common signs of this disease are including a change in breast shape, a red scaly patch of skin, a lump in the breast, dimpling of the skin, and fluid coming from the nipple. BC is the most common in females compared with males. In ancient times, it was considered to be rare until the 19th century when improvements in sanitation and control of deadly infectious disease resulted in dramatic lifespan increases. Previously, most women died too early to develop this disease [4]. There is evidence that developing signs of a relationship between irregular sleep, prevalence, and development of CA. In modern communities, night-time work is prevalent and growing. This exposure can be a concern to public health and lead to the ongoing rise in the risk of BC [5].

A group of hormones is regulated by the body, each of which has a specific role and any deficiency or lack of activity of these hormones can lead to certain problems in the functions of the body, which can also cause disease to develop. One of the most powerful hormones that affect body functions is melatonin (MLT). This hormone is excreted by the pineal gland in the brain which response to darkness and is controlled by the Suprachiasmatic nucleus (SCN). Melatonin is a small endocrine gland produced in darkness, thus usually at night. This hormone is synthesized by amino acid tryptophan and then converted to serotonin, and this finally causes to produce MLT, Serotonin is the precursor to MLT[6]. Melatonin has many biological functions that are regulating the sleep-wake cycle and are used for the short-term treatment of sleep disorder. The significant role of the

pineal gland is to diminish CA cell development. It leads to defending DNA in cells from damage [7]. It is important to note that MLT is produced not only by the pineal gland but also in the digestive tract, kidneys, and retina. High levels of this hormone have recently been found in the bone marrow cells [8]. MLT shows high lipid and water solubility is which promotes passage across cell membranes. Entry to different fluids, tissues, and cellular compartments (urine, saliva, milk, sperm, cerebrospinal fluid, and amniotic fluid) is acquired after release into the bloodstream [9]. The average MLT peak intensity is between 2:00 and 4:00 at night [10] and the release of MLT is reduced to almost the maximum stoppage between 7:00 and 9:00 in the morning. This decreased MLT state correlates with endogenous cortisol peak hours. Somewhere in the plasma, the average concentrations of MLT in plasma are between 60–70 pg/ml and the rise and decrease in MLT concentration [11].

For living biological systems, the Oxygen element is more important. In mitochondria, the cells are using oxygen to formed energy as adenosine triphosphate (ATP). The production of ATP process is produced by free radicals. Free radicals have duality properties, they do not affect normal levels but by overproducing their levels, they cause damage to cells via increasing the levels of oxidative stress [12].

Free radicals (FRs) are highly unstable molecules since they have unshared electrons. They have a negative effect at a higher stage because they cause several health problems**.** A variety of diseases has been associated with free radical damage within cells including CA, arthritis, atherosclerosis, Alzheimer's disease, and diabetes. FRs be toxic to the cells that generate them, or in contact with neighboring cells in a tissue or an organ. The cell produces free radicals and also degrades them. This is strictly required to prevent the damage resulting from a shape that is not regulated. Though, numerous intrinsic biochemical conditions and extrinsic situations and the cell's operation will because it loses control of the formation and regulation of FRs, this disparity in the formation and use of tissue FRs are referred to as oxidative stress. This results from a condition that leads to the equilibrium of reactive oxygen formation and the protection of antioxidants given by cells. This imbalance can cause damage to different human being's diseases, CA is one of those health problems that cause to production FRs in humans. There is a strong relationship between the levels of free radicals and antioxidants, increasing the level of free radicals in the body cause to decrease in the level of antioxidants [13, 14].

Antioxidants (AOs) are described as materials that can scavenge free radicals and decreases oxidation in body humans. AOs are compounds that inhibit oxidation. Oxidation is a chemical reaction that creates free radicals. Examples of AOs are ascorbic acid and thiols and in complex structures of competing AOs such as glutathione and enzymes [15].

Antioxidants can join with free radicals, providing them harmless and work against the group of chain reactions of radicals. Several evidences approved the association between each melatonin and total antioxidant capacity related to breast cancer [16].

In general, diet is the main important source to intake AOs. A significant number of studies in epidemiology have shown that individuals who eat a high-quality diet of certain plant foods (e.g. nuts), fruits, and vegetables are included in the decreased chance for the development of CA [17]. Dietary factors may also lead to avoiding breast cancer. In vitro experimental studies, for example, indicate that a high intake of AOs may reduce the risk of developing cancer. Besides, case-control studies have found that the consumption of fruits and vegetables is associated with a decreased risk of BC due to high AOs consumption [18].

The aim of this study is the determination of serum total antioxidants and melatonin levels on patients with breast cancer and compared with a healthy group at two different times 2:00 at midnight, and 9:00 in the morning.

2. LITERATURE REVIEW

This chapter describes the general information about breast cancer which includes classification and risk factors of breast cancer. Also, define, synthesis, and functions of melatonin describe in this chapter. At the end of this part, defines of each free radicals, oxidative stress, and antioxidants have also been illustrated.

2.1. Breast Cancer

Cancer of the breast is a heterogeneous disease related to different biological characteristics and clinical actions. The cells of the breast divide without control. BC is the most common type of CA in the female global. And it is responsible for higher carcinoma-related death in ladies worldwide [19]. The risk of BC increased significantly according to the light during darkness, Light exposure at night caused the disruption of circadian rhythms, and this cause to decrease in the levels of melatonin. This type of cancer is an oncological disease and one of the most public human neoplasms made up of ¼ of all cancers in women. It is related to the western lifestyle [20].

Breast cancer has been diagnosed in the oldest century, it was discovered by some of the researchers in previous studies. Breast cancer dates have been recorded 1600 BC and these described on long papyrus that was 468 meters which included 48 surgical cases, among these cases included at least 8 breast diseases. The original papyrus was much older from about 3000 BC. This papyrus was discovered by Edwin Smith in1862 who was announced as a god in an old Egypt. Several studies described the early stages of this disease, Celzo was founded the small tumors can be removed but the big tumors could be removed by surgery. Malignant and benign tumors were distinguished by Hippocrates. The first risk factor of BC was recorded from London in 1791-1805 at Middlesex Hospital. Leonidus was the first person who did the surgery of the BC. This surgery was included in the removal of the CA and some skin tissue by cauterization and cutting [21].

Breast cancer is growing up and detection in five steps, depends on the use of the (TNM) system was abbreviated for tumor (T), lymph nodes (N), and metastasis (M). In the beginning, is called pre-cancerous (CA does not develop), in the second step CA can develop to the tissues of the fatty breast. Next CA grows to lymph nodes, after that, spread to the wall of the chest. Finally, it has developed from one organ to another, this step is known as metastasis [22].

Breast cancer can be classified into two subgroups according to the histological type and grade. The histological type refers to the developmental design of the tumors and the histological grade has been included in multiple, prognostic algorithms, identification to control BC treatment. Besides,

cancer of the breast is classified into two types according to the stage, development and size are malignant (spread) and benign (does not spread) [23].

Breast cancer can be classified according to the histological status as non-invasive and invasive carcinoma of the breast. In the breast, non-invasive carcinoma stays inside the milk ducts or lobules. In or outside the breast, it does not expand into or invade normal tissues but invasive carcinoma develops into healthy, normal tissues. Non-invasive carcinoma consists of each ductal and lobular carcinoma. First, invasive ductal carcinoma, this is the commonest kind of BC is in the milk ducts, which accounts for three-quarters of cases grows. It can break through and enter the fatty tissue of the breast through the duct wall. Second, invasive lobular carcinoma accounts for approximately 15 percent of this type of BC. It originates in the milk-producing lobules of the breast. Both can spread (metastasis) to other parts of the body [24, 25].

In general, BC special types are made up of more than 27% breast carcinomas and according to the World Health Organization (WHO) categorization identifies the existence of nearly 17 different histological special types. Histopathological analysis of BC is used to determine both of the two most significant malignants are histological malignant type and grade. Metastasis of the breast is the ability to spread tissues from one organ to another without control. Chemotherapy, immunotherapy, and radiotherapy are used to treat BC. Oxidant/antioxidant balance is recognized as playing a role in the initiation and progression of cancer [23, 26].

Ellis et al. and WHO-researchers performed a study about the classification of genetics and pathological tumors of the Breast and Female Genital Organs, they demonstrated that invasive ductal carcinoma is the commonest type of BC, and both Acinic cell carcinoma and Oncocytic carcinoma are very rare [27]. Rakha et al. performed a study about the evaluation of the immune-histochemical shape and the morphological appearances of breast carcinoma. They also showed that the significant type of BC was invasive ductal carcinoma [28, 29]. Their results can be seen in Table 2.1.

Table 2.1. Prevalence of historical types of breast cancer [24]

Historical Types of Breast Cancer	Prevalence (%) WHO (Ellis et al., 2003)	Prevalence (%) (Rakha et al. 2006,2008)
Invasive ductal carcinomas (IDC)	50-80	56.4
Invasive lobular carcinomas (ILC)	5-15	8.2
Apocrine carcinoma	< 4	-
Invasive cribriform carcinoma	0.8-3.5	0.6
Neuroendocrine carcinoma	2-5	-
Invasive micro-papillary carcinoma (IMPC)	< 2	-
Pure tubular carcinoma	< 2	4.4
Mucinous carcinoma	2	1.4
Medullary carcinomas	1-7	2.6
Invasive papillary carcinoma	1-2	0.4
Lipid rich carcinoma	< 1-6	-
Glycogen rich clear cell carcinoma (GRCC)	1-3	-
Metaplastic carcinoma of the breast (MCB	< 1	-
Secretory carcinoma	< 0.15	-
Adenoid cystic carcinoma (ACC)	0.1	-
Oncocytic carcinoma	few cases	-
Acinic cell carcinoma	few cases	-

Some important risk factors of BC are family history, Ecological factors (e.g. light at night), fatness, Hormone Replacement Therapy (HRT), late age at first pregnancy, early menarche, infertility, Female gender, age, late menopause, late breastfeeding, diet, alcoholism, late age at lactation, short sleep duration. Other hazard factors are radiation, higher educational level exogenous estrogens, and socioeconomic grade [30].

In modern life, due to technological development, the number of shift workers has increased. Many medical disorders are related to the work at night (i.e. Night shift work) these medical disorders including gastric, circulatory, and neural disorders [31]. Nowadays, the most risk factor that causes the breakdown of the circadian rhythm is night shift work, and this leads to BC growth by the International Agency for Research on Cancer (IARC). Night shift work is expressed as working at night outside of normal daytime. Exposure to light at night (e.g. night shift work) has a higher risk of breast malignant. Because light prevents the secretion and synthesis of MLT hormone from the pineal gland [32].

Many risk factors cause increase breast cancer but the most significant ones are chronic inflammation, this is another significant problem in CA treatment, particularly for chemotherapy and radiotherapy. Besides, Inflammation causes to lack of response of malignant cells to both chemotherapy and radiotherapy and it's also involved in angiogenesis and metastasis. Another risk

factor of BC is Reduced low-molecular-weight antioxidants levels, containing ″glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), lipids, glutathione S-transferase (GST), glutathione (GSH), lipoproteins, and antioxidative vitamins″ [33].

Verkasalo et.al. Performed a study to determine the relationship between the degree of visual deficiency and the prevalence of BC in Finland, they collected samples in women who have had a visual light deficiency and they noticed that the risk of BC depended on the levels of the visual light. As a result, they demonstrated the risk of this disease decreased continually by the degree of visual light deficiency and their observation was the risk of BC prevalence amongst the blind women was substantially decreased by about 50% totally [34].

Hye-Eun Lee et al. Investigated the effect of light exposure at night work on BC patients. The sample collection is taken from night-shift worker women and with a healthy group. Eventually, they demonstrated the risk of BC in the group of night worker were significantly higher than the control group, because of the exposure to light at night cause to decrease the levels of melatonin [35].

2.1.1. Estrogens and Breast Cancer

A common example of hormone-dependent distortion is BC. Estrogens (Es) are involved with the development and separation of the typical mammary organ and they interfere in the development of ductal expansion. In any case, there is significant evidence that Es are additionally mammary CA-causing agents. Besides, Estrogen deficiency decreases mammary CAs, and Es management stimulates BC development. In humans, there are many significant roles of Es that cause the development of BC including never having been pregnant, fatness, early-age of menarche, late-age of menopause, and hormonal usage after menopause. These are BC risk factors to be directly associated with Es. There is a strong relationship between Es and BC because it is considered that two-thirds of BC occur in post-menopausal women, compere with pre-menopausal women, post-menopausal women have higher estrogen levels therefore, their ovaries have ceased to be functional and circulating levels of Es are low. In addition to this, some drugs are also causing to increase in the levels of estrogens including selective- estrogen receptor modulators (SERMs) such as tamoxifen and its derivatives [36].

2.1.2. Sleep Duration and Breast Cancer

There is evidence that the association between breast cancer and sleep duration. McElroy et al. and Verkasalo et al. explained the relationship between sleep duration and the risk of BC. They have collected serum samples from Japanese women who had suffered from BC and those who didn’t have this disease as a control group, they identified that those women who slept more the 9 h a night had a

non-significant increased risk of BC compare to those who slept less than 9 hours and the control group had a lower risk of the BC than those women who suffered this disease [37].

2.1.3. Night Shift Work and Breast Cancer

Shift work is characterized as work essentially outside of ordinary daytime working hours. These days the quantity of shift workers has expanded because of industrial progress. Several environmental factors lead to the occurrence of BC in shift workers given in Figure 2.1. Shift work is joined by a more noteworthy occurrence of numerous therapeutic disorders, for example, circulatory, gastric, and neural disorders [31]. Shift work that comprises diurnal disorder has been categorized by the International Agency for Research on Cancer (IARC), consideration to BC in night workers has been developing. Light exposure at night defeats the production of MLT and night shift work has been related to an increased risk of BC development [38].

Many researchers approve that night-shift workers incline to have lower levels of MLT than those who work daytime because of their light exposure at night. Several studies have shown an association with BC and altered clocks, ladies who work unbalanced shift patterns, for example, nurses, have a developed incidence of hormone-related mammary tumor. Developed levels of a tumor may be superior for ladies who ongoing night-shifts before the first full-term of pregnancy [39].

Hansen et al. and Davis et al. have researched women in employments who work during the night compared with those in occupations, they finally showed that the risk of BC in women with night-work more increased than those who work in a day [40]. The raised BC risk in females involved in night-shift work was a reliable conclusion in epidemiological revisions showed in Denmark. Nighttime light exposure decreases the rhythmic excretion or synthesis of MLT from the pineal gland. MLT hormone has oncostatic action in human mammary tumor cells in values in, and also in investigational animal with breast carcinomas. MLT stimulates genomic strength, and may also have anti-metastatic and anti-invasive action [33]

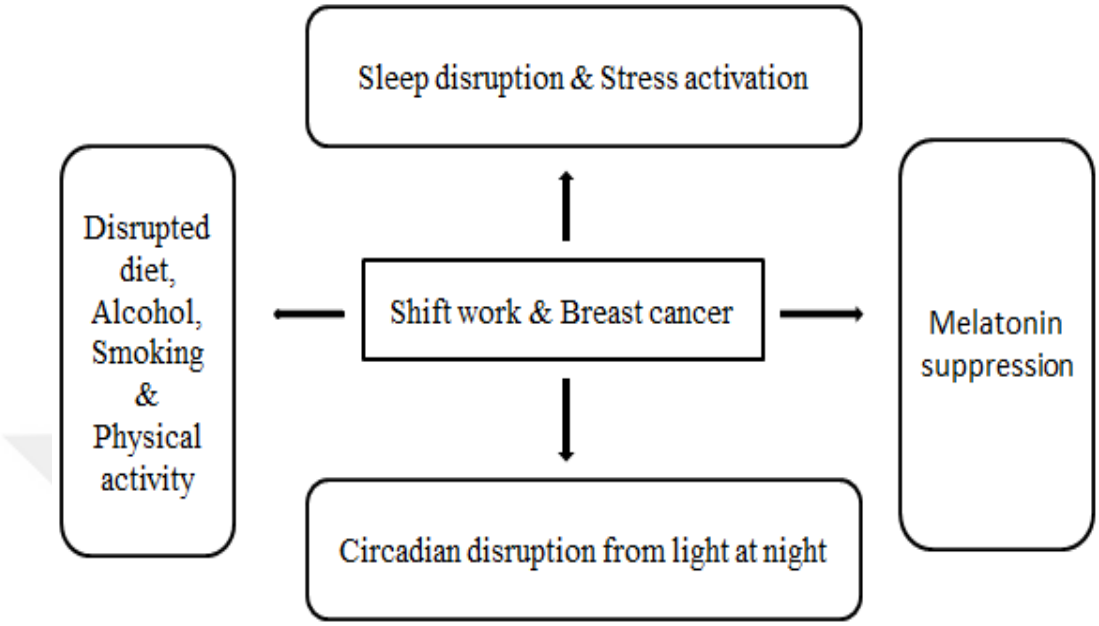


Figure 2.1. Different mechanisms of pathophysiology, behavioral and environmental factors that lead to the occurrence of shift worker breast cancer **[41]**

2.2. Risk Factors of Breast Cancer

The most prevalent form of cancer worldwide is breast cancer, and it is the second leading cause of death which can be preventable or non-preventable. Many significant risk factors that can cause to develop the risk of breast cancer are:

2.2.1. Menarche and the Menstrual Cycle

Early menarche has more effective in increasing the risk of BC. For each one year late in menarche, the risk declines by about 5 %. There is also evidence that all ages in menarche are concerned with the risk of BC, but this effect might be stronger in earlier menstrual cycle women. Besides, another significant risk factor that may affect BC is a short menstrual cycle interval which causes an increase in the risk of this disease [42].

2.2.2. Childbearing

Childbirth seems to have a double influence on the risk of BC; it is increased in the term directly after delivery, but this excess risk slowly declines and in the longer period, the influence of delivery is to defend against this disease. There is also evidence that the risk of BC in those older women who

have had their first full period of childbearing after age 30 around 25% higher than compared with younger women and those who have never been pregnant [43]

2.2.3. Breastfeeding

Breastfeeding duration is a significant factor; it's affected the BC. It will be better if this period is very long. Recent studies show that in less industrialized countries, the risk of BC is decreased because the breastfeeding duration can be very long compared with modern societies [44].

2.2.4. Menopause

Late menopause is one of those factors that cause develop the risk of BC. Those ladies who finish menstruating at an early age are at a lower risk of BC than those who experience menopause at a late age around 50 ages, by increasing each year older at menopause, the risk of BC will increase around 3% [30].

2.2.5. Diet

Nutrition is another important factor that has a direct effect on BC. Obesity can cause to increase in the risk of this disease. Nowadays, this risk factor is very common to compare to less developed countries related to fat intake in their foods in laboratory animals; some researchers have investigated this effect in rats. Finally, they showed that fatty foods are significantly caused to increase in BC development [45].

2.2.6. Alcohol and Smoking

Alcohol consumption is related to a modest rise in the development of BC and this risk grows approximately 10% per 10g alcohol consumed daily and by increasing this range about 30% of alcohol intake per day, this is related to an increase regularly around 30% in BC risk. Also, several studies demonstrated the association between cigarette smoking and the risk of BC, they showed both alcohol and smoking cause the development of the risk of BC [46].

2.2.7. Exercise

Many researchers have described adequate physical activity as linked to a lower risk of BC. They compared those women who have done the exercise a few hours per week have a lower risk than inactive women, they determined this decrease in risk about 30% between these two groups [47].

2.2.8. Family History

Domestic experience is another important factor that directly affected BC. Many studies have shown the first-degree relatives (i.e. daughters; mothers; and sisters) has more likely to develop BC risk than second-degree relatives (i.e. grand-daughters; grand-mothers; and aunts) [48].

2.2.9. Age

The occurrence of BC growth increases quickly with age. The older a woman is more likely to get this disease to compare to those who are at a young age. In general, this risk gradually increases after the age of 40. The increasing prevalence of BC among ladies in North America and Europe is about 3% by age 55, 5% by age 65, and 8% by age 75 [49].

2.2.10. Body-mass index (BMI)

Universal obesity becomes a public problem, Extra body mass has been occupied in around 5% of all tumors in the European Community 6% in women, and 3% in men which resembles an entire 45,000 female and 27,000 male tumor cases, containing 12,800 BCs per year. Obesity in ladies is recommended to an increased risk of evolving BC, while this does not implement similarly to teenage, pre-menopausal, and menopausal stages [50].

2.2.11. Inflammation

Chronic inflammation is a critical factor in CA progression. Adipocyte growth and cytokines storm during inflammation have an important role in BC. Adipocytes are endocrine cells that can synthesize a variety of hormones such as cytokines, leptin, estrogen, and adiponectin, these hormones have the ability to inflammatory cytokines, insulin resistance, and control immune function, these are associated with tumor initiation and propagation [51].

2.2.12. Environmental pollution

A recent study reveals that exposure to environmental pollutants increases the risk of BC in women. Several environmental factors cause to increase in the risk of this disease, including organochlorines. Moysich et al. showed that increased risk of BC was associated with higher serum levels of polychlorinated biphenyls (PCBs), and they noticed that women with higher levels who had never lactated, a significant form of the secretion of lipid-soluble organochlorines [52].

2.3. Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is an indolamine that is synthesized and secreted by the pineal gland in the brain. The chemical structure for this hormone can be seen in Figure 2.2. Melatonin is an abundant molecule in nature that is found in almost all living organisms. It is an amphiphilic characteristic of diffusion with a molecular weight of 232 g/mol. In 1958, American physician Aaron B. Lerner and his colleagues discovered and isolated this hormone by ethyl acetate from the bovine pineal gland in the brain. The cells of the Pineal gland are the ″pinealocytes″ that secrete melatonin and the ″glial cells″ which support neurons. Serotonin secretes by the pineal gland at night and then converted to melatonin by certain enzymes. Melatonin is known as darkness hormone [53]. It was also reported in each gastrointestinal tract, reproductive tract, retina, lens, liver, and skin, and spleen tissues. This hormone is found in animals, plants, and microbes. In the human brain, synthesized melatonin is regulated by the (SCN) of the hypothalamus [54-56].

Melatonin has diurnal variations in humans. MLT levels were significantly high in plasma compared with saliva. The intensity of MLT peaks was low before darkness but in the second half of the night between (2:00-4:00 at night), the intensity of MLT was gradually increased then it falls at (8:00-9:00 in the morning). On the other hand, Serum melatonin concentrations vary considerably according to age. Infants younger than three months of age secrete very little melatonin. In older children, melatonin secretion rises and becomes circadian, and peak nocturnal concentrations are highest at the age of one to three years (average, 325 Pg/ml) after which they steadily decrease by 10-15 % per decade. In normal young adults, the average daytime and peak nighttime levels are (10 and 60 Pg/ml) respectively. Furthermore, the level of melatonin was various during different seasons. In the winter, MLT levels were significantly increased during nighttime related to the summer [57].

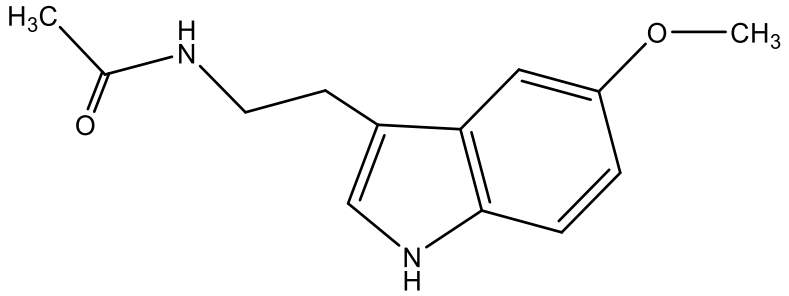


Figure 2.2. Chemical structure of melatonin

The functions of the pineal gland are to diminish the malignant cell's growth, it causes to protect DNA in cells from damage and it has a responsibility to regulate the neuroendocrine function. Biological rhythms reply to environmental hour signals to coordinate 24-hour in every body's tissue [58]. MLT has a variety of physiological functions, including controlling circadian rhythms (CRs), removing FRs, improving immunity, and inhibiting biomolecular oxidation in general. Melatonin deficiency is generally accepted to be closely linked to aging and age-related diseases such as Alzheimer's disease [59].

Melatonin has a wide range in the body called a ″chronobiotic″ because it is used for the circadian rhythm as a biochemical marker and synchronizes body rhythms. Circadian rhythms are biological processes that display endogenous and entrainable oscillation cycles that last approximately 24 hours [60, 61]. The electrical and metabolic activities of the SCN depend on the chronobiotic action of MLT. Melatonin is a significant modulator of the biological clock and organs containing the breast. MLT plays a significant character in controlling the body's biological clock. MLT cause increases therapeutic effects in CA patients and reduces the poisonous of chemotherapy. MLT is used as a biochemical marker for circadian level. Melatonin acts as AOs and free radical scavengers such as hydroxyl radicals (HO•). It also decreases the production of reactive oxygen species (ROS) and Nitric oxide (NO). Because of melatonin's antioxidant properties, they can be beneficial effects in delaying aging and in stopping age-induced degenerative sickness, for example ″Alzheimer's disease″ (AD), ″Parkinson's disease″ (PD), and neoplastic development. MLT has two significant metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK). The biological role of the MLT hormone in mammals is an antigonadotrophic activity [62]. The circadian rhythm disorders due to some of the environmental factors as shown in Figure 2.3.

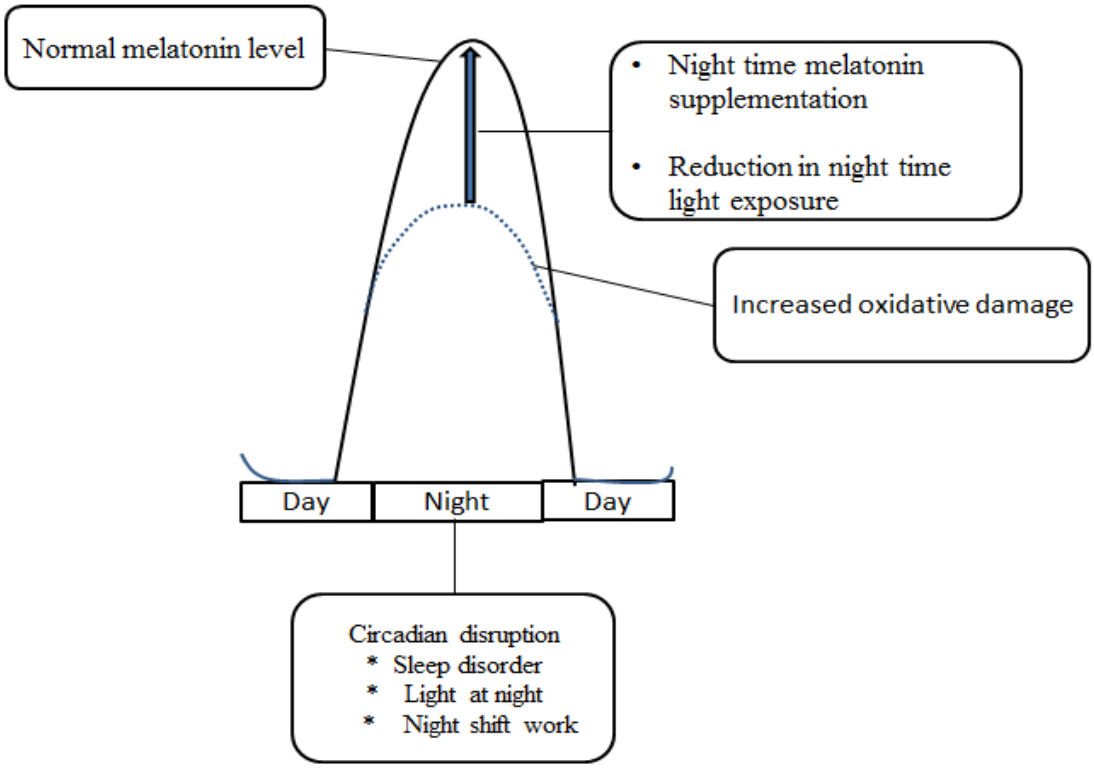


Figure 2.3. Medical considerations for melatonin administration [63]

Furthermore, Melatonin has a major role in neuroimmunomodulation in humans. It plays a significant role to keep safe in immunopathological states like inflammation and endotoxic trauma. The effect of the melatonin's antioxidant is to increase the amounts of SOD and GPx at the same time leads to a decrease in the malondialdehyde (MDA) levels, GSH, and NO activity. Melatonin's antitumor activities in BC can be divided as cytotoxic or cytostatic. Cytotoxicity in BC in a cell and malignant-specific behavior has been responded to by melatonin [64].

In humans, MT_1 and MT_2 are two types of melatonin receptors linking to the two membranes G-protein coupled receptors (GPCRs) acts as anti-malignant effects. Due to both MT1 and MT2 receptors, MLT can interact with different cellular kinases, these two types of melatonin-receptors are encoded by the activation of the melatonin membrane receptor (MTNR1A/B) [65]. The functions of these two types of receptors are controlling the SCN while the quinone reductase enzyme family (MT3) has been identified in birds and amphibia and helps a detoxification role in muscle, kidney, liver, and heart. The wide effect of MLT on breast cancer is cancer metabolism inhibition, acts as a scavenger of reactive oxygen species ROS, reduce toxicity, and genomic reactivity [66].

2.3.1. Synthesis and Functions of melatonin

Melatonin is the main biologically active substance synthesized from the amino acid tryptophan as a precursor and response to the sleep/wake cycle. In mammals, the pineal gland and retina are the primary sources of the synthesis of melatonin. In animals, Tryptophan is converted to 5-hydroxy-tryptophan by the enzyme tryptophan hydroxylase (TPH). Next, 5-hydroxy-tryptophan is converted to serotonin by the aromatic amino acid decarboxylase (AADC). Serotonin is converted to N-acetyl-serotonin by the N-acetyltransferase (NAT). In the final step, the N-acetyl-serotonin is converted to melatonin by the enzyme N-acetylserotonin-O-methyltransferase (ASMT) as shown in Figure 2.4.

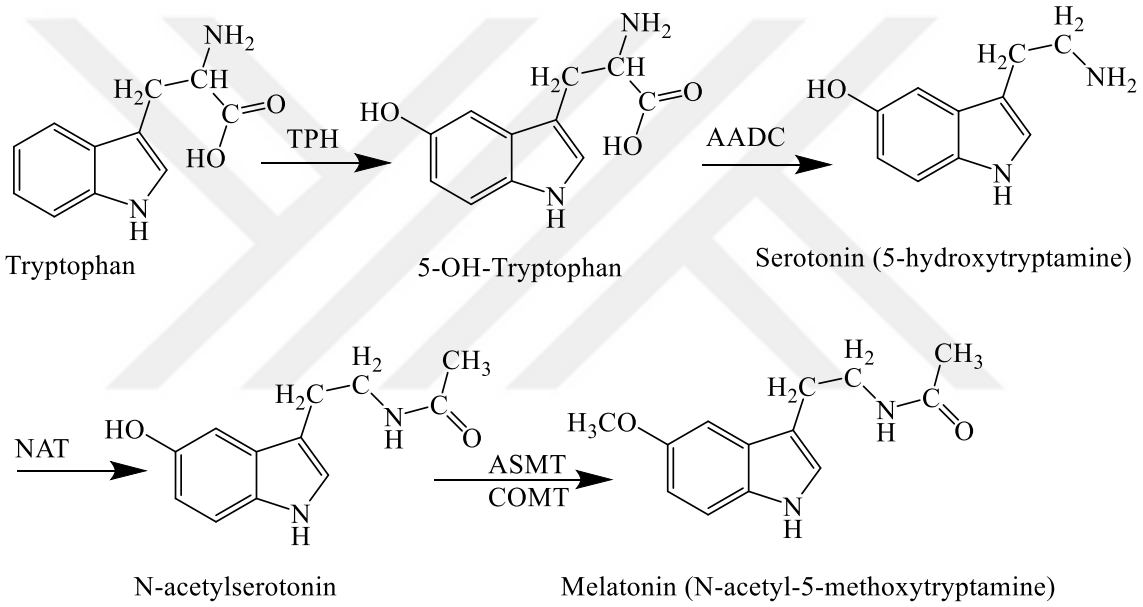


Figure 2.4. Biosynthesis of melatonin in animals

In plants, Tryptophan is synthesized due to carbon dioxide by the Shikimic acid pathway (it is a natural organic compound that is an important intermediate in the biosynthesis of amino acids like tryptophan). Next, tryptophan is converted to 5-hydroxy tryptophan by the enzyme tryptophan decarboxylase (TDC). Then, this is converted to serotonin by tryptamine 5-hydroxylase (T5H). Serotonin is converted to 5-methoxytryptamine by the N-acetylserotonin-O-methyltransferase (ASMT). Finally, 5-methoxytryptamine converted to melatonin by the N-acetyltransferase (NAT) as shown in Figure 2.5. [67].

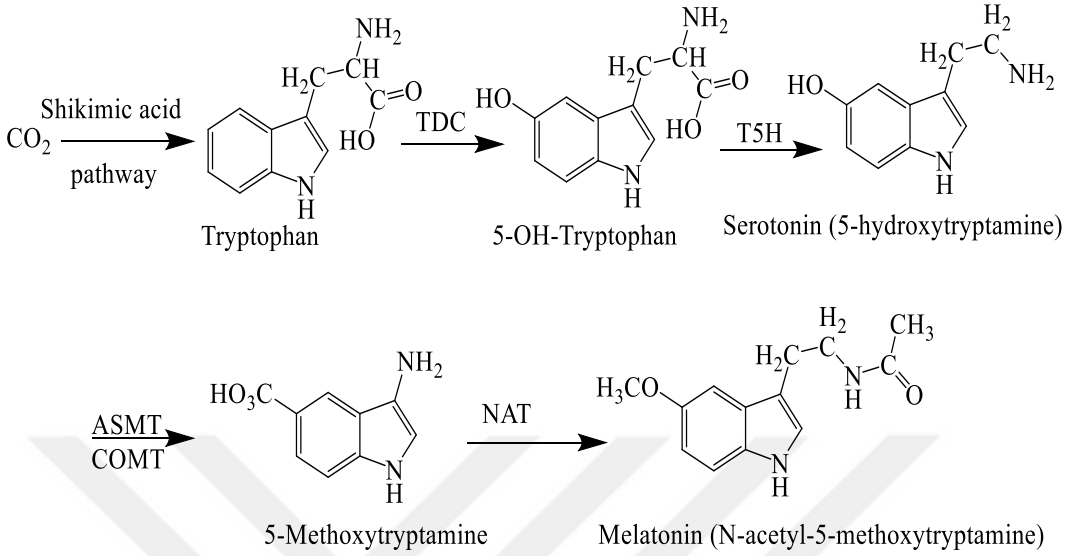


Figure 2.5. Biosynthesis of melatonin in plants

Melatonin has some important functions in the body as shown in Figure 2.6. such as free radical scavenger, it scavenges reactive oxygen species (ROS) and reactive nitrogen species (RNS) because it causes to stop the generation of free radicals [68]**.** Melatonin also acts as an antioxidant since it leads to a decrease in the effect of oxidative stress (OS) and increases antioxidant defenses [69]. MLT causes to protect cells and tissues from damage. MLT is amphiphilic because it can scavenge hydroxyl radicals (•OH) and reduce OS in both aqueous and lipid compartments of the body [70]. Serious side effects that restrict CA care can result from acute inflammation. Also, melatonin acts as an anti-inflammatory agent in radiotherapy [71]. The hypothalamic-pituitary-gonadal axis in humans is controlled by this hormone [72]. Melatonin detoxifies FRs as an efficient antioxidant and its existence is essential for cell and organ functions such as the reproductive system [73]. MLT encourages differentiation and decreases the proliferation of BC cells [74]. It has been shown that circulating melatonin levels are a good biomarker of circadian dysregulation and are associated with night shift work and light-at-night exposure.

The other functions of melatonin, including stimulating the production of natural killer cells (i.e. monocytes and leukocytes), MLT causes sleep regulation, development of immunity, acts as a multifunctional oncostatic agent, and modulation of CRs. In plants, melatonin continues to work in reducing oxidative stress as well as in promoting seed germination and development, improving stress tolerance, stimulating the immune system, and modulating circadian rhythms, a single MLT receptor has been found in land plants where it regulates stomatal closure on leaves [75].

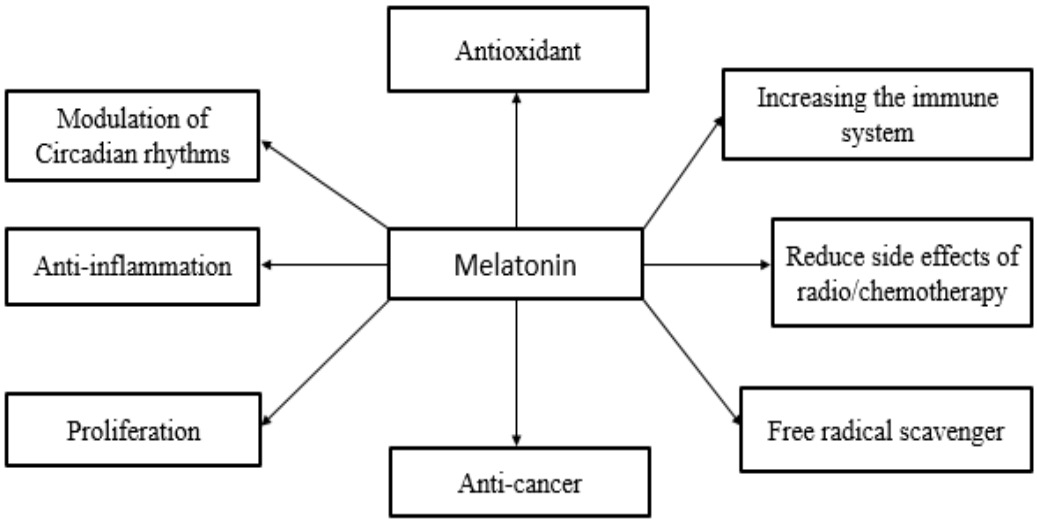


Figure 2.6. Functions of melatonin

2.3.2. Melatonin as antioxidant

Melatonin acts as an antioxidant through its action at two stages, first as a direct antioxidant via its ability to act as a free radical scavenger and second as an indirect antioxidant because of its ability to induce the activity of the main antioxidant enzymes. Melatonin is a powerful free radical scavenger because it can eliminate radicals such as hydroxyl radical (•OH), Hydrogen peroxide (H_2O_2), Peroxyl radical (LOO•), Singlet oxygen ($^1O_2$), and Peroxynitrite anion (ONOO-). Another important role of melatonin is to inhibit prooxidant enzymes such as nitrogen oxide (NO) synthetases and lipoxygenases which can weaken the response of the inflammation [76]. MLT can interact with free radicals since it has an electron-rich molecule and provides them stable compounds excreted by the urine. Diminishing oxidative damage in both lipid and aqueous environments occur due to melatonin. MLT is also able to protect DNA in the nucleus, proteins in the cytosol, and lipids in the cellular membranes from the damage of free radicals [77]**.** Furthermore, many researchers demonstrated that melatonin increases the expression or activity of antioxidant enzymes such as Glutathione peroxidase (GPx), Glutathione reductase (GRd), Superoxide dismutase (SOD), and Catalase (CAT) and this effect has been shown in rat brain cortex [78]**.**

2.3.3. Melatonin as Anticancer

The effect of melatonin as an anticancer can exert both direct and indirect. Direct anti-cancer action is exerted by inhibiting tumor cell proliferation and development, thereby impeding the propensity of healthy cells to become neoplastic, and by apoptosis, inducing ″cellular turnover″ and replacing tumor cells with healthy cells. MLT prevents the production of many human CA cell lines like breast, ovarian, liver, bladder, colon, and prostate CA cells. In pharmacological processes, Melatonin has cytotoxic effects on cancer cells while nocturnal, physiological levels convince the effects of oncostatic. Several studies have demonstrated that melatonin has anticancer properties when it uses with retinoic acid on MCF-7 hormone-dependent breast cancer cells which causes to complete halt in cell growth and a decrease in cell count by activation of apoptosis. Different studies have shown that Melatonin has oncostatic property because it halts the spread of cancer cells and causes to protect cells from DNA damage. MLT has able to defeat the collection of DNA adducts formed by carcinogens which cause permanent change and damage in DNA. This has been proved by rat experimental models of chemical carcinogenesis. Various researchers have shown that the anti-proliferative properties of MLT occur by cell cycle inhibition/blocking [79, 80]. Also, indirect anti-cancer action is exerted by decreasing the toxicity of chemotherapy, MLT keeps safe the relative lymphoid tissues and bone marrow against the effect of chemotherapy. Chemotherapy cause to decrease in the serum levels of melatonin [81].

2.3.4. Melatonin and Inflammation

Inflammation is the inherent response to harmful stimuli tissue such as infection and injury. Many significant factors cause to increase in inflammation such as environmental factors, epigenetic, and genetic. Inflammation may progress from initial to chronic stages which cause several chronic pathologies that have been related to different diseases, including tissue damage, encourage the staffing of additional leukocytes then cause organ failure and eventually to cancer [82]. The role of melatonin in inflammation is as an immune-modulator that shows both pro/anti-inflammatory agents. Several studies have demonstrated that melatonin is a prevalent anti-inflammatory molecule and has high clinical importance [83].

2.3.5. Melatonin and Immune system

The presence of an association between melatonin, pineal gland, and the immune system in birds and mammals, including humans, has been reported in several studies over the last 30 years. Melatonin may increase the production of cytokine. Cytokines are a variety of substances that are secreted by some immune system cells, such as interferon, growth factors, and interleukin. The notion that the pineal gland is linked to the immune system, especially the thymus, was first proposed in 1926 by Berman [84]. In an experiment in which kittens with pineal glands acquired from young bullocks were fed for two years, The subjects easily outgrow the controls in movement, size, intelligence, and resistance to neurogenerative disease, Berman commented that the animals are better protected against infectious diseases [85]. Several studies demonstrated that melatonin, the main endocrine compound of the pineal gland, has a potential regulatory role in communication between the neuroendocrine system and the immune system. Furthermore, the early studies on antitumor effects in animals and humans showed that melatonin could be an endocrine immunomodulator [86].

2.3.6. Melatonin and growth hormone

There is evidence that melatonin plays a significant role in regulating the release of growth hormones. Attanasio et al. studied the effect of melatonin on growth hormone (GH) secretion in rats, they found that MEL injections have a marked inhibitory effect on GH secretion. Smythe & Lazarus and Sorrentino et al. investigated that normal rats or blind rats were kept in consta nt darkness, showing that the secretion of MLT increased. Increased melatonin secretion shows decreased bodyweight, decreased length of tibia1, and decreased pituitary GH content [87]. Melatonin antisomatotrophic activity is, however, controversial. Injected hamsters with MLT exhibit increased body weight. MLT injections increase serum growth hormone levels in male hamsters every evening. The analysis of the facts available to man is even more fascinating. While idiopathic GH deficiency has been correlated with the absence of circadian rhythms (no association between endogenous nocturnal rhythms has been found in normal healthy individuals) [88].

2.3.7. Role of the Pineal Melatonin on Plasma Glucose Regulation

The Suprachiasmatic nucleus has a major role in controlling plasma glucose in the body. Damaging the Suprachiasmatic nucleus causes reduction functions of the circadian rhythms and loss of the ability of the pineal-MLT glucose balance, this work has done in rats [89]. Also, in the active period (morning stimulation), glucose and cortisol levels don’t rise before the beginning of this period

and in the inactive period (night-time depression), blood pressure doesn't incline in this period [90]. Therefore, the circadian rhythm of melatonin has direct effects on reduces blood glucose, insulin secretion in diabetic rats. While this MLT effect on plasma glucose is little known in humans. A significant role of melatonin is delaying or preventing diabetic onset. According to a recent study, a small amount of 1 mg of MLT in postmenopausal women reduced insulin sensitivity and glucose tolerance when controlled throughout the day. In animal models, melatonin can also be used to suppress glucose [91].

2.3.8. Role of the Pineal Melatonin on Blood Pressure Regulation

It has been known that the relationship between MLT and blood pressure variation, blood pressure occurs during lack of sleep in humans. This relationship was established by Millar-Craig et al. [92]. This study demonstrated that the blood pressure level was peak during mid-morning then drop during nighttime but is started to increase before awakening. According to this study, Circadian rhythm plays a vital role in the regulation of blood pressure by decreasing hypertension [93]. There is evidence that daily nighttime MLT has been revealed to increase the nighttime drop in diastolic blood pressure in patients with diabetes type 1 [94].

2.4. Sleep duration and Melatonin

The duration of sleep has a significant effect on melatonin secretion, increasing the number of hours of sleeping cause to increase in the levels of melatonin. Several studies have investigated the link between sleep duration and the production of melatonin. these studies demonstrated that there is a strong relationship between the production of MLT and the number of hours slept, as increasing number of hours slept, the primary urinary metabolite of MLT is more secretion [55].

Wu et al. studied the relationship between the risk of BC and sleep duration. They found that with increasing sleep duration (more than 9 h), the risk of BC decreases. Besides, they also showed that those volunteers who slept 9+ h during the night had substantially higher levels of urinary MLT than those who slept fewer [95].

Wibke Bleicher et al. investigated the relationship of patients with insomnia (a sleep disorder) in postmenopausal women and the levels of melatonin. They observed that in patients with insomnia in postmenopausal women, MLT levels were substantially lower relative to the healthy community. Consequently, the risk of BC in patients was significantly higher than in the control group [96].

2.4.1. Effects of Light on Melatonin

Light is another important factor after sleep duration but this factor may be more effective in some individuals than others as shown in Figure 2.7. Several sorts of light's influence apply to the MLT proposition for the association between electrical power of BC and alteration of melatonin function. Light is more effective on the pineal function in humans and some individuals are more sensitive than others, light exposure at night can be perceived in individuals in the interior for 15 minutes, the brighter the light the greater the reduction in circulating melatonin [97]. Blue-green light is the most influence in decreasing the production of melatonin while red-light has little or no effect, and in the nighttime, the MLT levels are much higher than the daytime, the standard melatonin rhythm in individuals has features that may apply to the risk of the breast cancer [98].

Benot S et al. investigated light exposure at night resulted in a clear decrease in melatonin. They demonstrated the age of the volunteers was significantly effective in decreasing the levels of melatonin, The levels of this hormone were diminished in older individuals compared with younger individuals and day-night differences were also studied, produces melatonin at night much higher than the day [99].

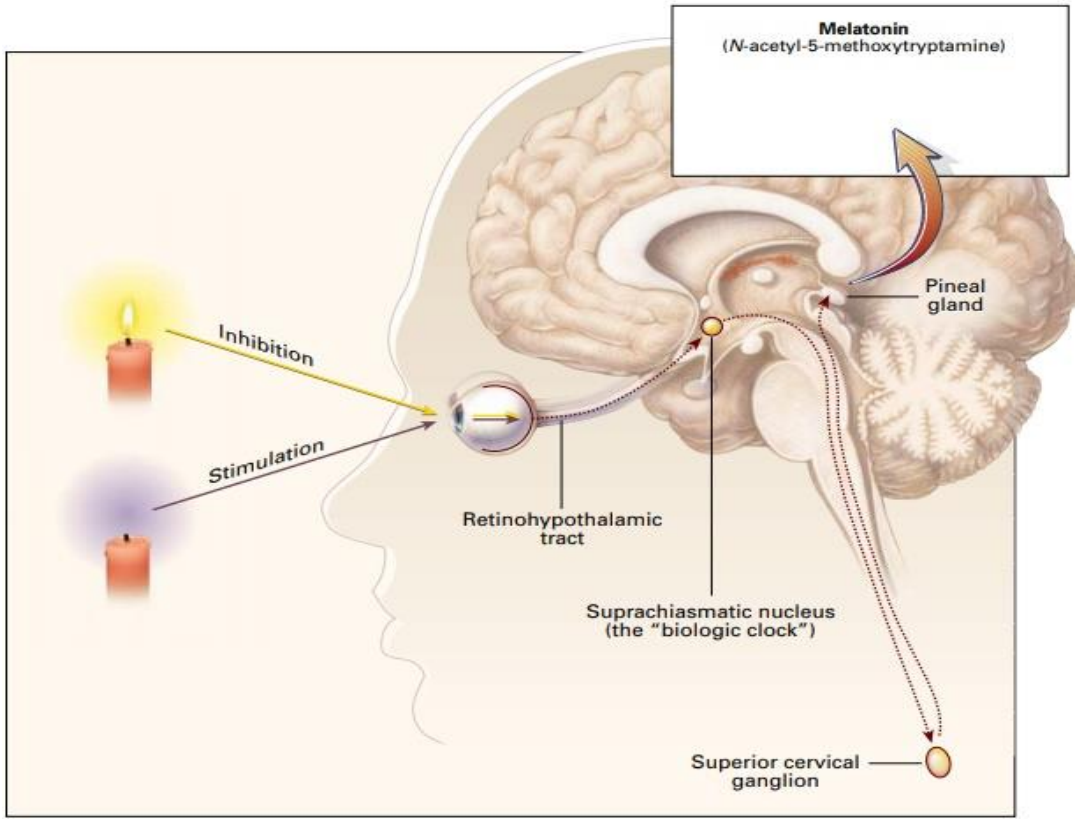


Figure 2.7. Effect of light on melatonin excretion [100]

2.5. Melatonin deficiencies

Lack of melatonin has been essentially examined in the pineal gland, cerebrospinal fluid saliva via assessing the metabolite 6-sulfatoxymelatonin, urine. The nighttime melatonin peak is commonly detected to decrease throughout aging [101]. The case of decreased melatonin levels, which surpass those detected throughout normal aging have been frequently defined in neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and different kinds of decrepit dementia [102]. Melatonin rhythm is almost stopped in many cases of individuals due to the consequence of SCN degeneration. On the other hand, sleep disorder and decreased secretion of melatonin are occurred due to tissue destruction in the SCN or the pineal gland [103]. Besides, many diseases and disorders in the body have been described due to decreases in melatonin levels that are not related to apparent SCN degeneration that is metabolic and neurological disorder such as migraine, certain types of CA, diabetes type 2, and general insulin resistance. Sleep disturbance is related to the changed CRs of cortisol [104].

Leproult et. al. studied the effects of sleep disturbance on the hypothalamic-pituitary-adrenal axis and found that the levels of cortisol have been elevated in the evening during total or partial sleep disturbance. Elevated cortisol levels have been found among BC patients. This destruction of cortisol profiles has been found to expect previous death with metastatic breast cancer [105].

2.5.1. Melatonin and Alzheimer

Alzheimer's disease (AD), an age-related neurodegenerative disorder with gradual memory loss and extensive cognitive impairment, is characterized by aggregated β-amyloid (Aβ) extracellular senile plaques and hyper-phosphorylated tau protein intracellular neurofibrillary tangles. In patients with AD, reduced levels of melatonin in serum, and cerebrospinal fluid (CSF), and loss of MLT diurnal rhythm were reported. In several studies, melatonin plays a major role as an antioxidant and neuroprotector in aging and AD. A recent study has shown that β1-adrenergic receptor mRNA has disappeared, both MAO-A function and gene expression have been upregulated in AD patients, indicating that dysregulation of noradrenergic innervations and depletion of serotonin, the precursor of melatonin, may be responsible for the loss of melatonin rhythm and decreased melatonin levels in this disease [59, 106].

2.5.2. Melatonin and Parkinson’s disease

Parkinson's disease (PD) after Alzheimer's disease is the second most common neurodegenerative disorder (the breakdown of the cells in the brain). In the substantia nigra and striatum, it is marked by the gradual depletion of dopamine. These Neurodegenerative disorders are caused primarily by oxidative damage. Melatonin has been successfully tested in both PD models in vivo and in vitro. Sleep-wake boundary disorder is rising in Parkinson's disease. There is growing evidence that melatonin is an endogenous, non-toxic, antioxidant drug without known side effects. In PD patients, indolamine should be regarded as a valuable agent as a co-treatment with traditional therapies [107].

2.6. Free Radicals

Free radicals are extremely unstable particles produced by biochemical oxidation/reduction reactions (redox reaction) that take place as a part of normal cell breakdown and in the progression of free radical-mediated diseases including diabetes, tumor, rheumatoid arthritis, renal, circulatory system, infectious, inflammation, and neural diseases. Free radicals are extremely unstable because of the presence of unpaired electrons in their outmost orbit. FRs are compounds with an odd number of electrons that might be zero, negative, or positive charge. Based on the particle located at its center, the radical can be defined as nitrogen, carbon, oxygen, or metallic placed radical. Indeed, FRs are playing a dual role in the human body as both beneficial and deleterious species because they engage in normal physiological processes at low concentrations for example the body obtained energy by oxidizing carbohydrates through both anaerobic and aerobic processes, each protein and fats cause to generate free radicals. but Overproduction of FRs can responsible for tissue damage and lead to a decrease in antioxidant levels due to increasing the levels of oxidative stress [108].

Free radicals reduced the action of enzymatic antioxidants in breast cancer. These FRs are detached and/or deactivated in vivo by a series of antioxidants. Emerit et al. investigated that FRs can induce widespread tissue damage, reacting with macro-molecules, for instance, proteins, nucleic acids, and membrane lipids. Also, Senem et al. showed that the levels of free radical particles are organized by several cellular defense components, containing nonenzymatic mechanisms (GSH, vitamin C, and vitamin E) and enzymatic mechanisms (SOD and GPx). Nowadays, the role of FRs in the origin of different diseases has been commonly determined. The cell produces FRs and also damages that which is firmly required to avoid the damage resulting from uncontrolled creation. Free radicals play an important role in the body's human, in killing the microorganisms by body process or signal conversion and in cell signaling. Moreover, they have a damaging effect since they damage the oxidative activity of the organism which causes numerous health difficulties [15, 109].

2.6.1. Reactive Oxygen Species and Reactive Nitrogen Species

Free radicals are also called reactive oxygen species (ROS). The most important oxygen-containing FRs in many disease states are singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical (HO•), and superoxide anion ($O_2•^-$). Reactive oxygen species (ROS) are generated in metabolic and physiological mechanisms, and dangerous oxidative reactions might be occurring in organisms, which eliminate them due to the non-enzymatic and enzymatic anti-oxidative processes [110].

Reactive oxygen species are always generated inside the cell as a consequence of the mitochondrial electron-transfer mechanisms. They contain a large group of oxygen derivative small particles that comprise non-radicals and radical types. Oxygen and nitrogen reactive species are formed as a result of regular aerobic metabolism in animal kinds. They are proficient in removing a hydrogen atom from poly-unsaturated fatty acids in membrane lipids to start lipid peroxidation. Hydroxyl radical and its consequent radicals are the most dangerous ROS and they are essentially responsible for the oxidative damage of biomolecules. Oxygen and nitrogen reactive species have a negative role at top-levels in the body because they cause damage to DNA and other biomolecules and play an important role in genetic instability, influencing cell cycle development, cell repair, and the type of cell death (apoptosis, senescence, or autophagy). In the processes of cell transformation, differentiation, and cell proliferation, free radicals are also important and may be useful for assessing the inflammatory response of the tissue. Finally, these radicals have been implicated in tumor growth, angiogenesis, immune response, and invasive and metastatic tumor cell potential concerning carcinogenesis. The carcinogenic process carcinogenesis of oxygen and nitrogen reactive species has been shown in Table 2.2. [111].

Table 2.2. Representative diagram of the oxygen and nitrogen reactive species [13]

Reactive Oxygen Species (ROS)	**Reactive Nitrogen Species (RNS)**
Superoxide anion $O2 \xrightarrow[\text{Oxidase}]{\text{NADPH}} O_2^{\bullet -}$	Nitric oxide $\text{L- Arginina} \xrightarrow[\text{NOS}]{\text{L-Arginine}} NO^{\bullet}$
Hydrogen peroxide $O2^{\bullet -} \xrightarrow[\text{SOD}]{O2^{\bullet -},\ O_2} H_2O_2$	Peroxynitrite $NO^{\bullet} \xrightarrow{O2^{\bullet -},\ O_2} ONOO^{\bullet}$
Hydroxyl radical $H_2O_2 \xrightarrow{\text{Fenton reaction}} {}^{\bullet}OH$	Nitrogen dioxide $NO_2^{\bullet}$
Hydroperoxyl radical $O_2 \xrightarrow{H^{\bullet}} HO_2^{\bullet -}$	Nitrous anhydride $NO^{\bullet} \rightarrow N_2O_3$

2.6.2. Hydroxyl Radicals

There are two main reactive oxygen species in living organisms, superoxide radical ($\bullet O_2^-$) and hydroxyl radical (•OH), which are continuously formed in a process of reducing oxygen to water. superoxide radical is a diatomic oxygen radical anion that contains the superoxide ion. In 1934, Haber and Weiss discovered hydroxyl radicals known as the Fenton reaction [112]. Hydroxyl radicals are biologically active free radicals that comprise one unpaired electron and a single proton that generated by two major reactions are"Fenton reaction" and " Haber-Weiss reaction" as shown in. In the Fenton reaction, hydrogen peroxide and ferrous ion are converted to the ferric ion and hydroxyl radicals. Also, In the Haber-Weiss reaction, superoxide and hydrogen peroxide are convert to oxygen and hydroxyl radical as can be seen in Table 2.3. Hydroxyl radicals are beneficial in normal levels because they occur in different types of environments such as the atmosphere, biological systems, and natural waters but in case of excessing levels, they become harmful in the body for example damage in DNA cells and reduce protein disulfide bonds, specifically fibrinogen, resulting in irregular spatial configurations of their unfolding and scrambled refolding. In many illnesses, such as atherosclerosis, cancer, and neurological disorders, the effects of this reaction are observed and can be avoided by the action of non-reducing substances [113].

Table 2.3. Generation of hydroxyl radicals

Fenton reaction	Haber-Weiss reaction
$Fe^{3+} + \bullet O_2^- \rightarrow Fe^{2+} + \bullet O_2$ $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$	$\bullet O_2^- + H_2O_2 \rightarrow O_2 + OH^- + \bullet OH$

2.6.3. Oxidative Stress

The human body is constantly exposed to potentially toxic oxidative stresses throughout a lifetime, which may occur from exogenous factors such as diet, environmental contamination, like tobacco smoke and exhaust emissions, or ionizing radiation [114]. The term oxidative stress (OS) is a typical word used to characterize a genuine contrast in creatures between the age of ROS and the cancer prevention agent barrier process, causing a state of a potential hazard. In other words, Oxidative stress is an imbalance between the production of free radicals and antioxidant defense which can lead to tissue damage as shown in Figure 2.8. Oxidative stress causes damaging reactive biochemical species that are existent in tissue as a result of the regular cellular oxidative process of both exogenous and endogenous

compounds. High levels of OS harm the human body because it leads to damage to DNA, cells, and proteins. The levels of antioxidant capacity depend on the levels of each OS and oxidants [115].

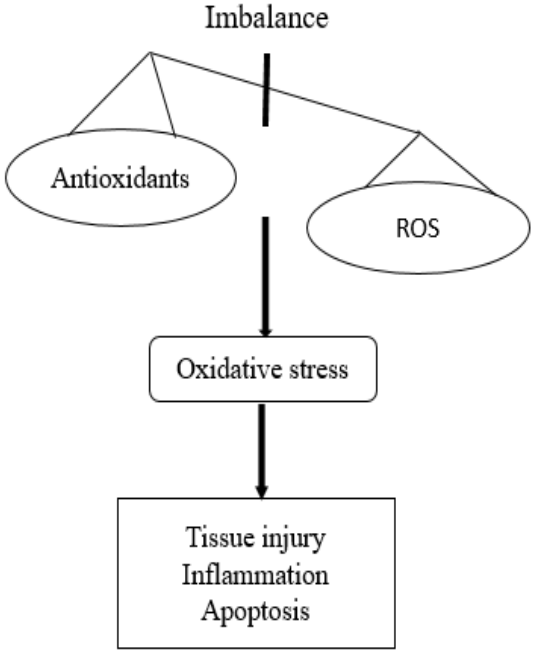


Figure 2.8. Effect of Oxidative stress on the body

Oxidative stress has a negative effect on the body because they cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis, and the excess generation of oxygen free radicals can cause carcinogenesis. Carcinogenesis is a complex process that involves a sequence of cellular and molecular events that promote the transformation of a normal cell into a cancer cell [111].

Derya Erten et al. performed a study of OS in patients with BC. Serum samples were taken from patients with BC and the control group, they showed that patients with BC have significantly increased OS compared with the healthy group [116].

2.7. Antioxidants

An antioxidant is a substance that protects cells against free radicals and cause delays or prevents oxidation. Antioxidants are segments of diet that are involved with DNA and cell protection from oxidative damage. They are referred to as mopping up free radicals because they cause reducing oxidative DNA damage and oxidative stress [117, 118]. The amount of DNA damage was significantly decreased by antioxidants such as Superoxide dismutase (SOD), Catalase (CAT), and Glutathione (GSH). A variety of nutrients, including antioxidant vitamins such as carotenoids, tocopherols, vitamin C, and flavonoids, are obtained from fruits and vegetables [119].

Antioxidants are divided into two classes are non-enzymatic and enzymatic antioxidants. non-enzymatic antioxidants consist of the exogenous and endogenous. Exogenous antioxidants, including trace elements,vitamins and carotenoids, flavonoids and fatty acids which are obtained from the diet and endogenous antioxidants such as melatonin, uric acid, glutathione, and coenzyme Q10 are products of the body's metabolism. Enzymatic antioxidants such as Glutathione peroxidase (GPx), Glutathione reductase (GR), CAT, and SOD are found in human milk as shown in Figure 2.9. Antioxidants are vital parts in sustaining an appropriate healthy body which play a significant role in the reduction or delays oxidation of the substrate and they attach with FRs, providing them safe and stop FRs from forming chain reaction [120].

In general, water-soluble antioxidants react with oxidants in the cell cytosol and blood plasma while lipid-soluble antioxidants protect cell membranes against lipid peroxidation. These compounds may be synthesized in the body or acquired from the diet [70]. The functions of antioxidants are to increase the free radical scavenging activity and decrease the harm caused by free radicals. The most significant role of an antioxidant involves scavenging free radicals that containing reactive nitrogen species (RNS) and reactive oxygen species (ROS). Besides, they also play a major role in the use of melatonin as a radio-protector [121].

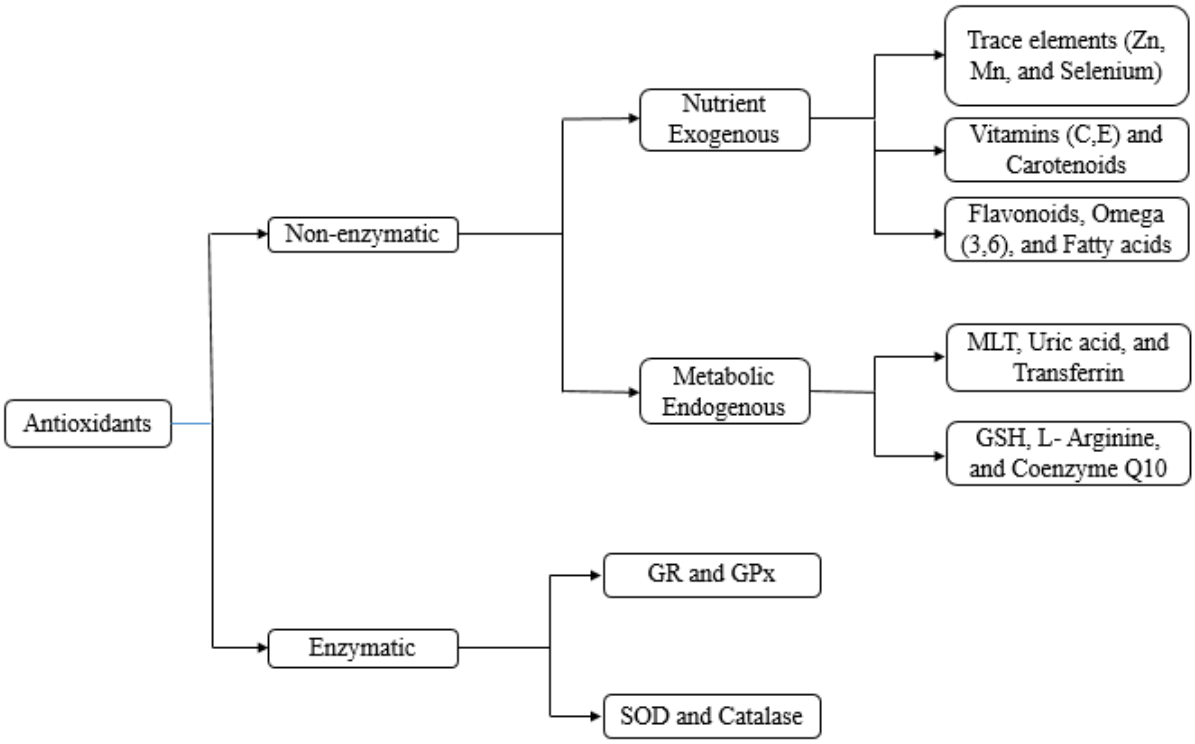


Figure 2.9. Classification of antioxidants

2.7.1. Antioxidants in plants

Plants are protected from destructive reactions due to the effective antioxidant defense due to scavenging the ROS because there are many sites in plants to produce ROS, including chloroplast, mitochondria, and cytosol. Plants have developed various phytochemicals and enzymes as antioxidant protection to sustain growth and metabolism. Antioxidants are formed in leaves and defend the plant from damage by reducing free radicals [122]. The best example for plants that act as antioxidants are flavonoids, Flavonoids are polyphenolic compounds found naturally in many vegetables and fruits [123]. The main source of natural antioxidants is fruits and vegetables because they comprise substantial levels of biologically active mechanisms that communicate health assistance outside basic nutrition [124]. Fruits and vegetables are major sources of vitamins such as ascorbic acid (vitamin C) which acts as an antioxidant. Yim Tong Szeto et.al measured the ascorbic acid content of fresh vegetables and fruits and total antioxidant capacity. They took different types of fruits, including orange, strawberries, kiwi, and pineapple, and compared with Apples, bananas, plums and pears they showed bananas, plums, pears, and apples contain small amount of ascorbic acid and indicated the antioxidant capacity of vegetables decreases rapidly and after fragmentation [125].

2.7.2. Functions of antioxidants

Antioxidants have many important functions in humans and plants, including defeating the formation of FRs and detoxified them, stopping chain initiation, and breaking chain propagation to form FRs, assist to decrease the prevalence of oxidative stress-induced damage [126], and protect the immune cells from OS are controlled by antioxidants. It stimulates the growth of normal cells, helps fight age-related molecular degeneration, protects cells against premature and abnormal aging [127].

In humans, the levels of antioxidants play a vital role in the prevention and development of carcinogenesis. Several studies have shown that low consumption of AOs or low blood levels of AOs raises the risk of various diseases, low dietary intake of vegetables and fruits cause to increase the risk of cancer. Vegetables and fruits are sources of several nutrients, including antioxidant vitamins for example flavonoids, carotenoids, and vitamin C [119, 128]. AOs preserve the function of membrane lipids, nucleic acids, and cellular proteins [129].

In plants, regular consumption of fruits and vegetables has been predictable as reducing the risk of chronic diseases. AOs play a major role to reduce the rate of oxidation of fats and oils [130]. Water-soluble antioxidants in plants such as reduced GSH and ascorbate are accumulating to millimolar concentrations in chloroplasts. Metabolites of these two antioxidants are scavenging ROS, Antioxidants cause to increase photosynthesis which involves the green pigment chlorophyll and generates oxygen as a byproduct [131].

2.7.3. Deficiency of antioxidants

Antioxidants play a crucial role to protect cells from damage by scavenging free radicals, prevent or delay oxidation and it can decrease the level of oxidative stress in the body. On the other hand, deficiency of antioxidants causes to increasing production of free radicals due to the increase of oxidation which can responsible for oxidative DNA damage and this may lead to diseases such as breast cancer. Antioxidant deficiencies lead to increasing the levels of oxidative stress. Furthermore, excess free radicals in the body are related to environmental factors, including pollution, infection, inflammation, cigarette smoking, and radiation [120].

2.7.4. Total Antioxidants

Total Antioxidant Capacity (TAC) is described as a parameter cause to increase the activity of all the AOs in blood humans that use to measure the constant rate of single antioxidants against oxidants [132]. TAC is used to measure the overall antioxidant capacity of the body. The overall activity of antioxidants and antioxidant enzymes is summarized by the TAC parameter. The depletion of total antioxidant capacity caused by oxidative stress is eliminated by the release of stock organ AOS, primarily from the liver and adipose tissue, and the induction or activation of antioxidant enzymes. TAC drop due to the degradation of antioxidants at a later stage of oxidative stress [109].

Total antioxidant capacity levels depend on the rate of each oxidative stress and oxidants, both oxidative stress and oxidants cause decreasing the activity of total antioxidants, it means by increasing the rate of each oxidative stress and oxidants, the levels of TAC will decrease. Many risk factors affected the levels of total antioxidant activities, including, weight, age, and night-shift work. Night workers for example nurses have lower levels of antioxidants but higher levels of OS. Exposure to light at night cause to decrease in the TAC levels. Several methods have been developed to measure the total antioxidant potential of human serum or plasma due to the difficulties of separately measuring each antioxidant component and the interactions between different components [133].

Kely R. C. Teixeira et al. performed a study about the effect of night-shift work on antioxidant defense and oxidative stress on breast cancer patients compared to day workers. For this purpose, blood samples were taken from two groups, one was taken from patients with BC, and two was a healthy group. Finally, their results showed that the night worker has lower levels of antioxidant defenses and has higher levels of oxidative stress damage compared with the control group [134].

Benot S et al. performed a study about the determination of melatonin concentration that contributes to the total antioxidant status (TAS) of human serum in the day and nighttime. They collected serum samples from healthy volunteers of different ages (ranging from 2 to 89 years). Their results showed a clear association between melatonin and TAS in human serum, both have peak values around 1:00 hr. at night [99].

.

3. MATERIAL AND METHOD

This chapter describes how collected samples and which instruments are used to analyze the samples. Also, it has illustrated that how prepared samples and which method was used for the detection of samples.

3.1. Chemicals and reagents

Human serum melatonin, Butylated hydroxytoluene (BHT), Sodium Carbonate (Na_2CO_3), Methanol for HPLC, gradient grade (99.9 %), Ethanol (96 %), Ethyl acetate, n-hexane, Dimethylformamide (DMF), Sodium hydride (NaH), Benzyl chloride, Human serum total antioxidants, Reagent 1 (Buffer solution), Reagent 2 (ABTS radical cation), Standard (Trolox equivalent that is water- soluble vitamin E analog).

3.2. Sample Collection

Blood samples were collected from 33 breast cancer patients and 22 controls in Hiwa Cancer Hospital in Northern Iraq. This study was approved by the Centre ethical committee Written informed consent from each participant was subsequently obtained. The options were given to them to withdraw from the research according to their wishes. Five milliliters of Venus blood for melatonin and five milliliters for total antioxidants were drawn from patients and controls at two different hours (2:00 at night and 9:00 in the morning) by venipuncture using a plastic syringe with a gauge of 21 stainless needles. Blood was separated in a plain polyethylene tube, left to clot at room temperature and the serum was separated from the whole blood by centrifugation 1500 x g for 10 minutes. The separated serum was stored at (-20 ^{0}C) until analysis.

Collected samples from women in both of the breast cancer cases and controls of different ages who lived in different cities. The controls were relatives with cases. All cases were diagnosed with breast cancer. The controls were selected from healthy women who had no smoking and alcohol use and who did not regularly use drugs. Breast cancer cases for this study were as follows: 23 breast cancer cases have received more chemotherapy treatment and 10 cases have received few chemotherapy treatments. 8 of the cases had no surgical intervention and 25 of them had surgery for their one breast. The patient group has had in different ages and stages of their disease. Some of the breast cancer patients have had other diseases such as blood pressure, thyroid disease, diabetes mellitus, liver disease, rheumatoid arthritis, and kidney disease. All the control group individuals were relatives with patients. This is means that all the control group persons have had a family history.

3.3. Preparation of serum sample

3.3.1. For HPLC

First, 100 µL of BHT and 100 µL of Na_2CO_3 were added to 1.3 ml of the serum sample and vortexed for 2 min. and then 2 ml of methanol and 1 ml of ethanol were added to the solution and centrifuged at 4420 rpm for 1 hour and separated serum from the solution. Next, added 200 µL of ethyl acetate and 200 µL of n-hexane, vortexed, and centrifuged at 1300 rpm for 5 minutes and the supernatant was taken from the sample and then performed the extraction and dry the sample with nitrogen gas. After, added 300 µL of DMF solvent and small amounts of NaH then incubated for 30 min. in the refrigerator. After incubation, added 50 µL benzyl chloride to the samples and incubated for 1 hour in the refrigerator. Finally, the serum melatonin analysis was performed by HPLC.

3.3.2. For ELISA

Pipette 18 µL of serum sample and 300 µL of the reagent 1 (Acetate Buffer 0.4 mol/L and PH 5.8) into a cuvette and mixed well and then read absorbance (A1) after 30 seconds at 660 nm. Next, added 45 µL of the reagent 2 (ABTS radical cation Prochromogen solution 30 mmol/L) to the solution and mix well and then read absorbance (A2) after 5 minutes at $37C^0$. Rel Assay Diagnostics ELISA Kit was used in this study and the results were compared with the Trolox equivalents antioxidant capacity (TEAC). Finally, the serum samples were analyzed for total antioxidants by ELISA with the commercial kit (Mega Tıp Sanayi ve Ticaret Limited Şirketi | Gaziantep / TURKEY), and then the results were compared with the (TEAC).

3.4. Instruments

3.4.1. High-performance liquid chromatography (HPLC)

HPLC is one of the most important types of chromatography techniques that is very sensitive and useful for qualitative and quantitative analysis. In this study, UV-visible HPLC was used for the determination of serum levels of melatonin. The mobile phase was consist of the flow rate, wavelength, and column temperature. It was prepared for the preparation of serum samples. The mobile phase was 300 ml of acetonitrile (CAN) + 50 ml of potassium dihydrogen phosphate (KH_2PO_4) (35+ 65, v/v %, pH 4), 15 ml of dimethyl sulfoxide (DMSO), 175 ml of distilled water, and the flow rate is 1.0 ml/min. Chromatograms were read at 250 nm and the injection volume was 50 µL. The retention time was 3.52 min. Liquid chromatographic system (Shimadzu, Kyoto, Japan) consists of the following:

2x LC-20AD pumps, 1x DGU-20A5 degasser, 1x SIL 20A autosampler, 1x CTO-10AS VP column oven, 1x SPD-M20A DAD system, 1x RF-10AXL FLD system and 2x detectors connected in series. These devices were connected via a communication module (Model CBM-20A) and controlled by a Shimadzu LC Solution workstation. A Shimadzu Shim-pack VP-ODS column (150L×4.6) was also be used.

3.4.2. Enzyme-linked immunosorbent assay (ELISA)

ELISA is an immunochemical test that detects and measures antibodies in the blood. It is very sensitive and requires minimal reagents that are used for quantitative and qualitative analysis. In this study, Total antioxidants were measured by using a commercial ELISA kit which includes a microtiter plate with wells coated with serum total antioxidants as antibodies. $ABTS^{\bullet+}$ method is based on the bleaching of the characteristic color of a more stable blue-green ($ABTS^{\bullet+}$) by antioxidants. The assay is calibrated with a stable antioxidant standard solution which is traditionally named Trolox Equivalent that is a vitamin E analog. ELISA test is based on the principle of competition for the binding sites of the antibodies coated on the wells between an unknown amount of total antioxidants in the sample and a fixed amount of enzyme-labeled antigen. Incubation times, shaking processes, and temperatures were set according to the directions provided in the kit. Finally, absorbance was recorded at 660 nm.

3.5. Statistical Analysis

Statistical analysis and plot drawing were performed using SPSS statistics (Version 22) and Excel program (2013 for Windows 2010) software. For both paired and unpaired samples, a student's t-test was used to explore statistically significant differences where needed and these values were used for comparison between the graphs. P-value = 0.017 and 0.001 for the Paired T-test in the patient and control groups at 2:00 and 9:00 respectively. P-value < 0.01 for the unpaired T-test in both of the patient and control groups. A statistically significant decreased between these two groups was observed.

4. RESULTS AND DISCUSSION

Cancer of the breast is the most common type of different cancers in women worldwide. It is related to some significant features, sleep disorder is one of those features that cause the development of the risk of this disease due to a decrease in the level of melatonin in the body. Breast cancer is a very difficult disease because many factors have an effect on it such as sleep duration, family history, chemotherapy and the stage of this disease, obesity, environmental pollution, and lifestyle. There is a strong relationship between sleep duration and the risk of breast cancer. On the other hand, there is evidence that decreasing total antioxidants levels are also effective in decreasing the risk of breast cancer and there is an association between night-shift work, total antioxidant capacity, and breast cancer development.

In this thesis, performed a case-control study to assess the association between serum levels of melatonin and total antioxidants with breast cancer at two different times (2:00 in the night and 9:00 in the morning). This study determined the levels of both melatonin and total antioxidants in breast cancer patient and the control groups at the same time and illustrated some important features that cause to decrease in this level. Sleep duration, receiving chemotherapy treatment, age of the patient group, and stage of breast cancer are the most significant factors that directly affected in increasing and decreasing of the levels of both melatonin and total antioxidants according to our findings.

Many risk factors cause to decrease in the levels of melatonin such as age, stage and chemotherapy treatment, obesity, Sleep duration, and exposure to light at night. Breast cancer cases who participated had their blood collection at a very stressful time, this may cause to decrease in the levels of melatonin and total antioxidants.

The results show that these factors affect in increasing or decreasing the levels of melatonin in patients with breast cancer as well as healthy individuals. We observed the following results comparing the highest levels of melatonin with the lowest.

4.1. The levels of melatonin in the breast cancer patient and control groups

Figure 4.1. and Figure 4.2. show the levels of melatonin in breast cancer cases and controls according to the number of samples respectively. In these figures, the blue color has represented the hour of sleeping at 2:00 at night and the green color has also represented the hour of awake 9:00 in the morning. According to our results, the levels of this hormone were high at 2:00 at night and low at 9:00 in the morning in both the cases and control groups but this level more significantly higher in the control than the patient group.

The figure 4.1. shows the maximum and minimum levels of melatonin in the breast cancer patient group, the maximum level of this hormone at 2:00 at night was equal to (39.3 Pg/ml) in the sample (31) and the minimum level was equal to (20.8 Pg/ml) in the sample (9) as well as the maximum level at 9:00 in the morning was reached (15.7 Pg/ml) in the sample (29) and the minimum level was (7.1 Pg/ml) in the sample (9).

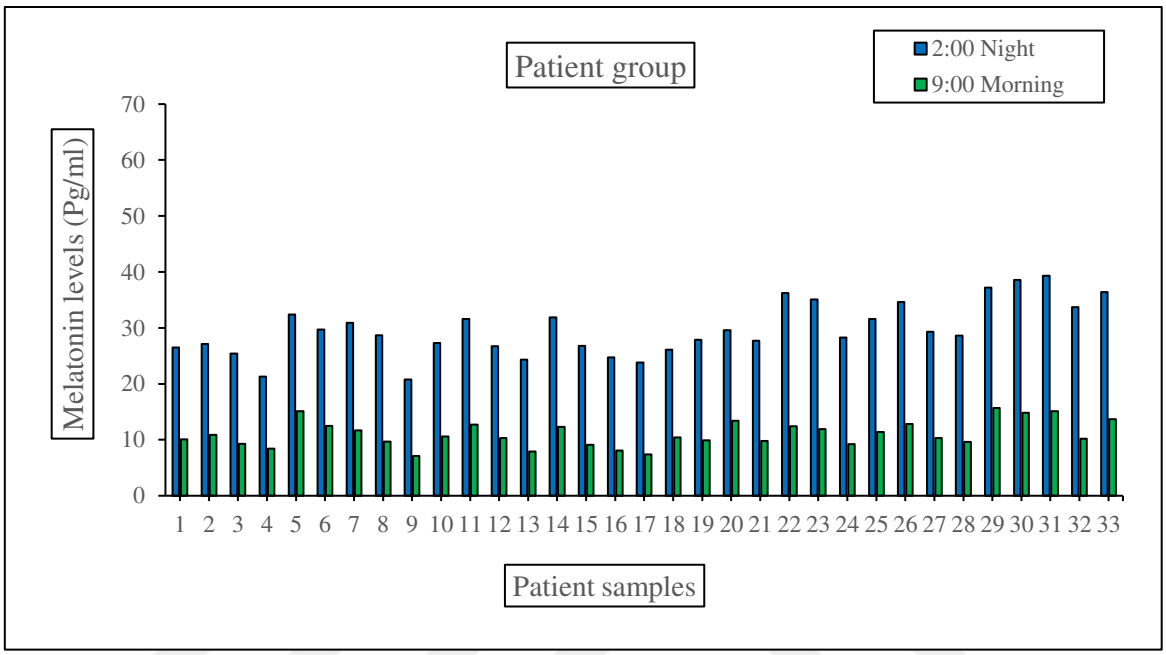


Figure 4.1. Melatonin levels in the breast cancer patient group

The figure 4.2. shows the maximum and minimum levels of melatonin in the control group, the maximum level of this hormone at 2:00 at night was equal to (65.9 Pg/ml) in the sample (22) and the minimum level was (38.7 Pg/ml) in the sample (12) as well as the maximum level of this hormone at 9:00 am was reached (19.4 Pg/ml) in the sample (8) and the minimum level was equal to (10.4 Pg/ml) in the sample (2).

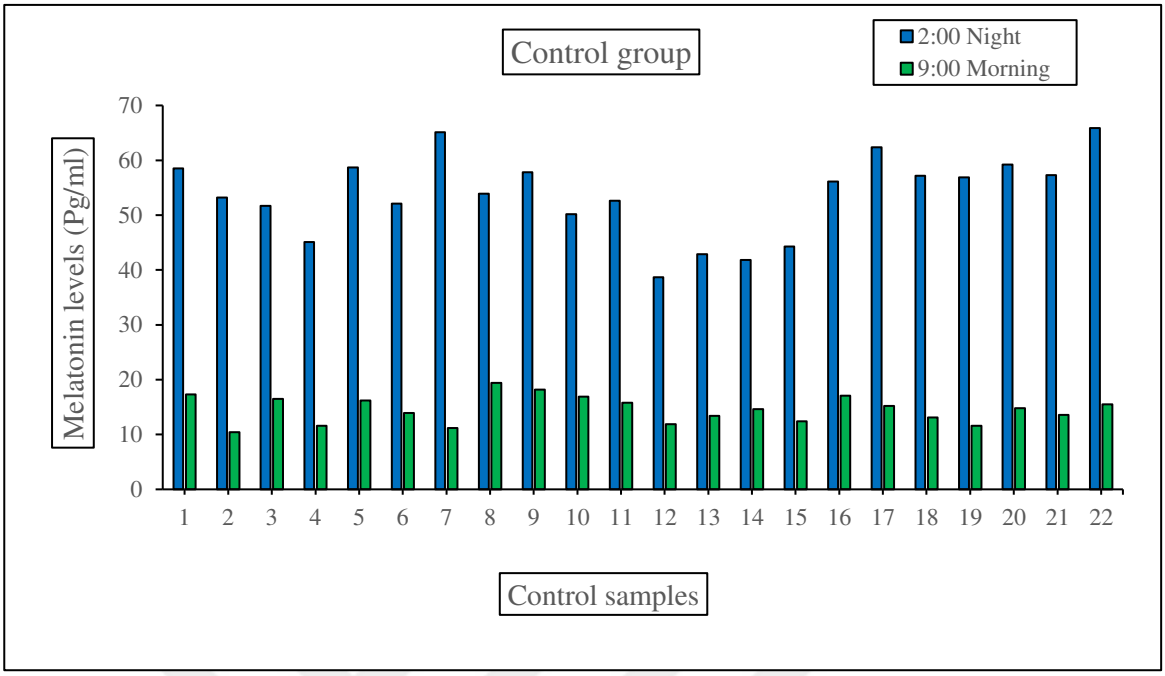


Figure 4.2. Melatonin levels in the control group

In summary, the levels of melatonin in both case-control study were high at 2:00 at night and low at 9:00 in the morning but this level in some of the samples was low in nighttime this may be related to those factors illustrated below.

4.1.1. The levels of melatonin in the breast cancer patient and control groups according to age

There is a strong relationship between melatonin levels and the age of the patient and control groups. The serum melatonin levels vary considerably according to age because those samples who were younger, this level was significantly high compare to those who older according to our results. Figure 4.3. and Figure 4.4. illustrate the relationship between melatonin levels and the age of the patient and control groups respectively. The results of this study showed that by increasing the age, the levels of melatonin were decreased. The samples have been arranged from younger to older in both groups. The age of the patient group was started from 30 to 63 years and the control group was from 25 to 50 years.

The figure 4.3. shows the maximum and minimum levels of melatonin in the patient group, the top level of melatonin in this group at 2:00 at night was reached (39.3 Pg/ml) in the sample (31) and then reached to minimum level was (20.8 Pg/ml) in the sample (9) as well as the high level of this level at 9:00 am became in the sample (15.7 Pg/ml) in the sample (29) and the minimum was (7.1 Pg/ml) in the sample (9).

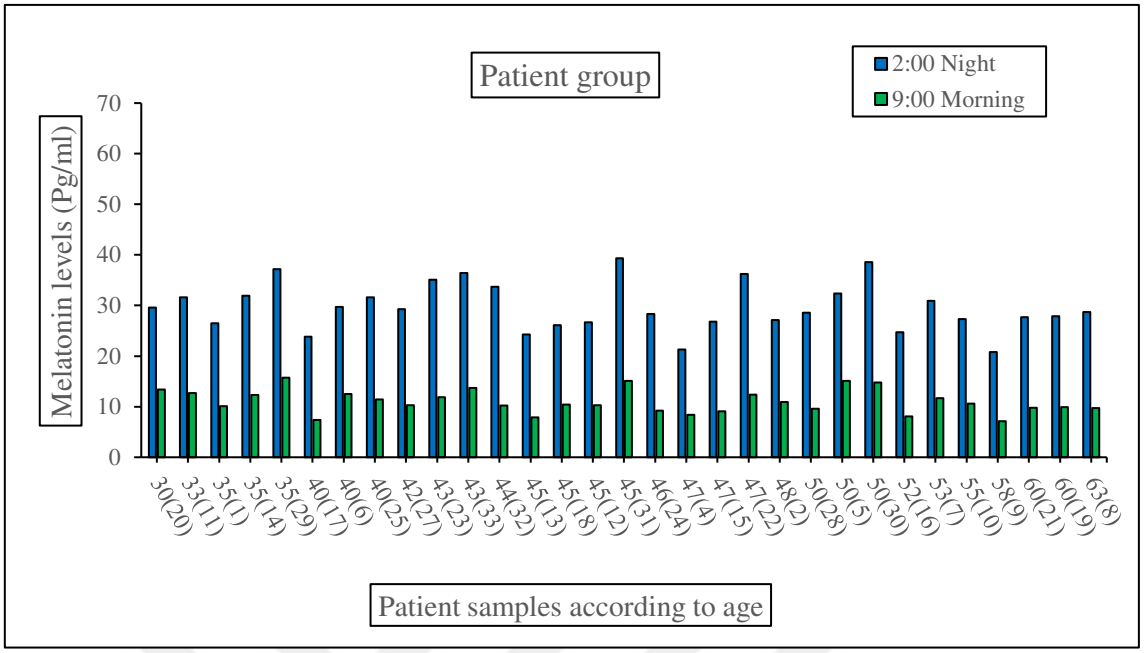


Figure 4.3. Melatonin levels in the breast cancer patient group according to age

It is important to notice that some of the samples at 2:00 and 9:00 hours in both patient and control groups have different peaks while they had in the same age, this may affect some of the important factors such as the stage of this disease, chemotherapy treatment, and sleep duration. The table below shows some important factors which may cause different increases in the levels of melatonin while the patients have in the same ages. These changes in the melatonin levels in this group have been shown from Table 4.1. below:

Table 4.1. Illustrates the patient group with the same age but different in melatonin levels

No.	Age	Melatonin levels	Stage	Chemo	Marital status	Family history	Sleep duration	BMI	Exercise
1	35	low	4	more	Married	no	few	obese	no
14	35	middle	3	more	married	no	more	obese	no
29	35	high	3	more	unmarried	yes	few	obese	no
17	40	low	4	more	unmarried	no	More	normal	no
6	40	middle	2	more	Married	yes	more	Obese	no
25	40	high	3	more	unmarried	yes	more	normal	yes
23	43	Low	2	few	married	yes	more	obese	no
33	43	high	3	more	married	yes	few	obese	no
13	45	low	3	more	married	yes	more	obese	no
18	45	middle	1	few	married	yes	few	obese	yes
12	45	middle	3	more	married	yes	more	obese	no
31	45	high	1	few	married	yes	more	obese	no
4	47	low	4	more	unmarried	no	few	obese	no
15	47	middle	1	few	married	yes	more	obese	no
22	47	high	4	more	married	no	more	obese	yes
28	50	low	1	few	married	no	few	obese	no
5	50	middle	2	few	married	yes	few	normal	no
30	50	high	1	few	married	no	more	obese	no

The figure 4.4. shows the maximum and minimum levels of melatonin in the control group. in this group, the maximum level at 2:00 was equal to (67.1 Pg/ml) in the sample (3) and the minimum was (38.7 Pg/ml) in the sample (12) as well as the top-level at 9:00 was (19.3 Pg/ml) in the sample (3) and then declined to (9.9 Pg/ml) in the sample (10).

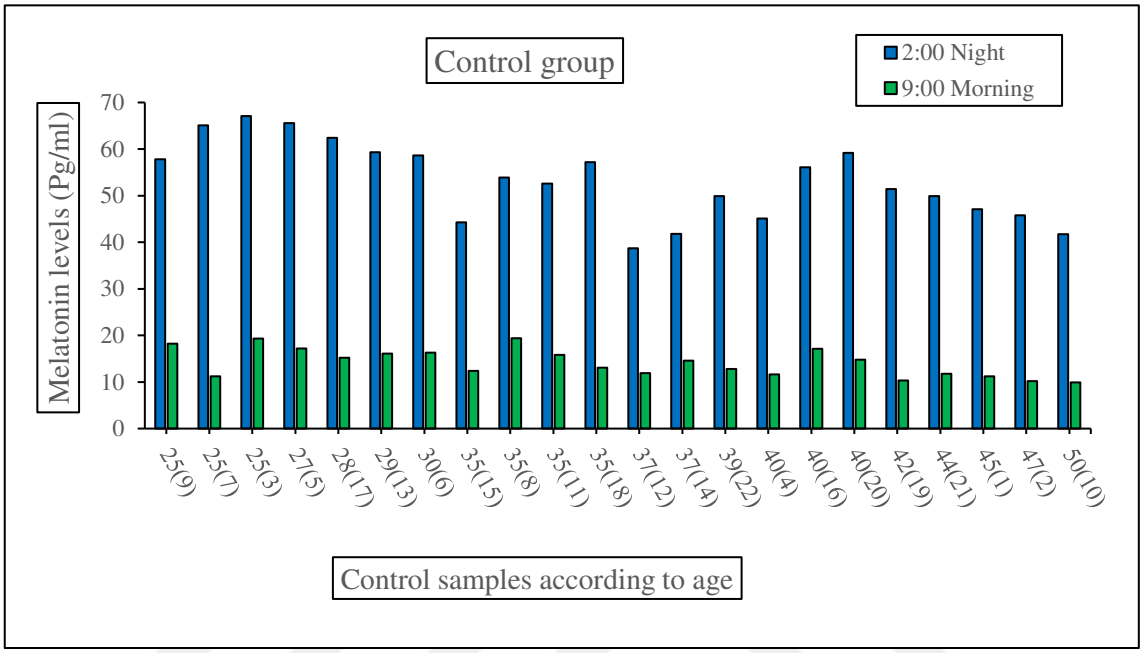


Figure 4.4. Melatonin levels in the control group according to age

4.1.2. The levels of melatonin in the breast cancer patient group according to the stage and chemotherapy treatment

Chemotherapy has more effect to decrease the levels of melatonin. This level may increase or decrease by receiving few or more chemotherapy treatment. In this study, it is important to notice that all patients have received chemotherapy treatment. Having received a few or more chemotherapy treatments was also be effected on this level. The effect of chemotherapy on melatonin levels according to the stages of this disease was also shown in this study.

The figure 4.5. illustrates the relationship between the levels of melatonin, few and more chemotherapies in breast cancer patients. It has shown that the level of melatonin much lower in those patients who have received more chemotherapy treatment compare to those who have received few since chemotherapy has a direct effect on decreasing the level of this hormone according to our findings.

In this figure, the patient samples have arranged according to the increase in stages and more received chemotherapy treatment. Melatonin levels at 2:00, the high level of this hormone was equal to (39.3 Pg/ml) in the sample (31) and the low value was (21.3 Pg/ml) in the sample (4) as well as these values at 9:00, the top level was equal to (14.8 Pg/ml) in the sample (30) and the minimum was (8.4 Pg/ml) in the sample (4).

The samples (30,31,28, 15, 18, 8), the levels of melatonin at 2:00 and 9:00 hours are decreased from left to right according to this figure while they all have in the same stages in this disease, this may be related to the age and sleep duration. In the sample (31), the patient was 45 years old and she slept more compare to the sample (8) who was 63 years old and she had few slept.

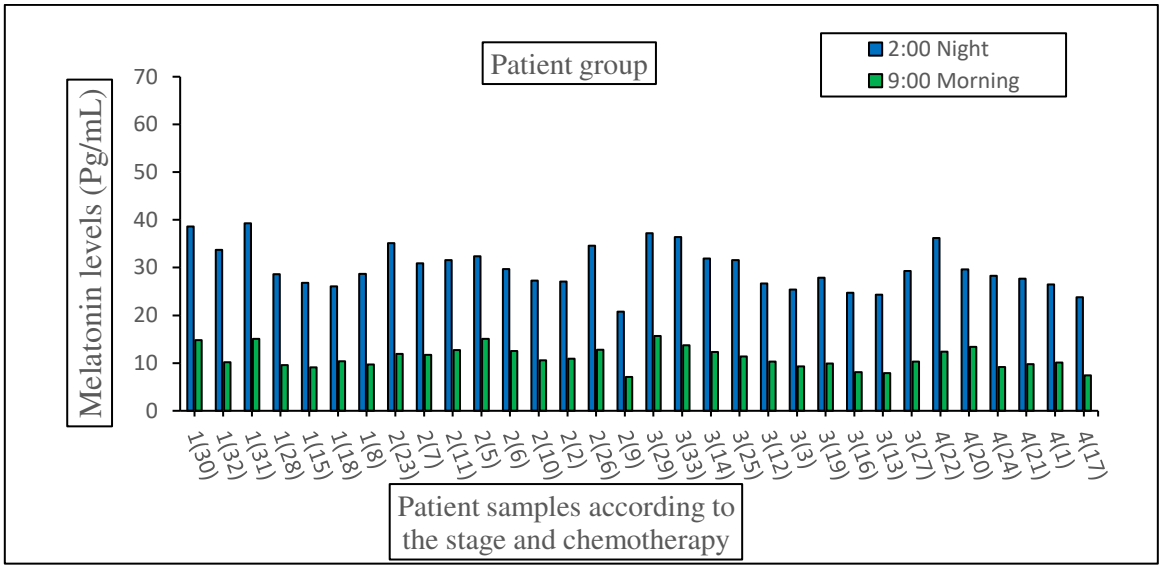


Figure 4.5. Melatonin levels in the breast cancer patient group according to the stage and chemotherapy treatment

The figure 4.6. shows that those patients who had in different stages of their disease and who have received few or more chemotherapy treatment. The levels of this hormone were decreased from left to right according to the increase in their stages in both different hours. The (TNM) system has been used to determine the stage of breast cancer. 7 patients were classified as stage one, 9 patients as stage two, 10 patients as stage three, and 7 patients as stage four.

In stage 1, the maximum level at 2:00 was reached to the maximum level (38.6 Pg/ml) in the sample (30), and in stage 4, it was reached to the minimum value was (21.3 Pg/ml) in the sample (4). This level in stage 1 at 9:00 was reached to the maximum value (14.8 Pg/ml) in the sample (30) and was decreased to a minimum level was (8.4 Pg/ml) in the sample (4)

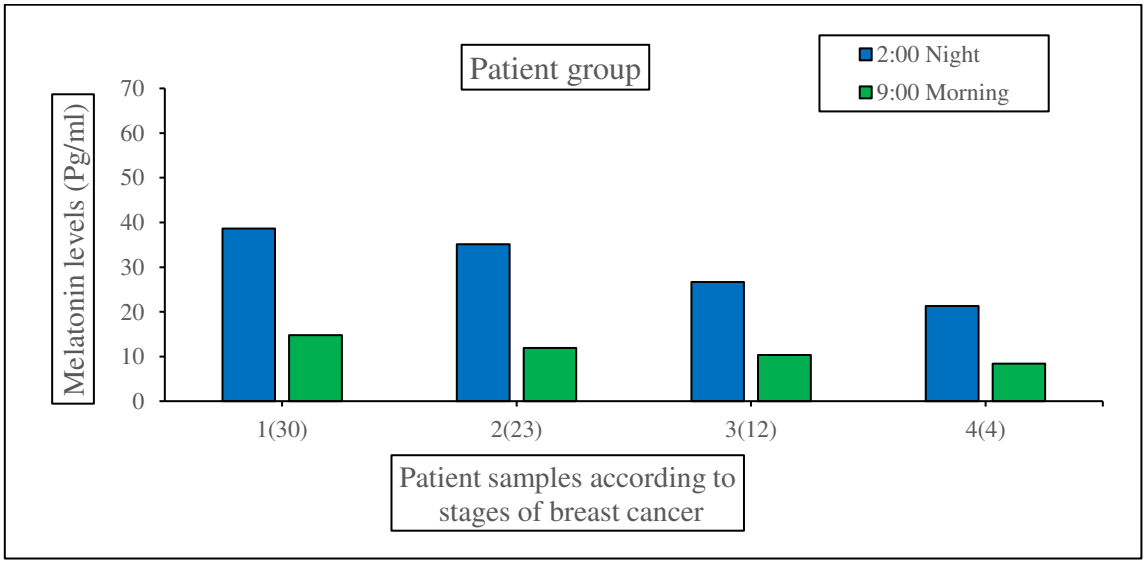


Figure 4.6. Melatonin levels in the patient group according to the stage of breast cancer

In summary, our study showed that received more chemotherapy treatment cause to decreasing the serum levels of melatonin. On the other hand, by increasing the stage of breast cancer, the levels of melatonin were also be decreased as shown in the figure above. The table below shows some important features that have a negative role to decrease the levels of pineal melatonin hormone. Those patients have the same stages in their disease but different in receiving chemotherapy treatment. Sleep duration is another important factor that causes to increase or decrease in this level. These factors have shown in Table 4.2. This table is also shows different levels of melatonin in the patient group while they have the same stages in breast cancer disease.

Table 4.2. Illustrates the patient group with the same stage but different in melatonin levels

No.	stage	Melatonin levels	age	Chemo.	Marital status	Family history	Sleep duration	BMI	exercise
30	1	high	50	few	married	no	more	obese	no
32	1	middle	44	few	married	yes	more	obese	no
31	1	high	45	few	married	yes	more	obese	no
28	1	low	50	few	married	no	few	obese	no
15	1	low	47	few	married	yes	more	obese	no
18	1	Very low	45	few	married	yes	few	obese	yes
8	1	low	63	few	married	no	more	normal	no
23	2	high	43	few	married	yes	more	obese	no
7	2	middle	53	more	married	no	few	obese	no
11	2	middle	33	more	married	yes	few	normal	no
5	2	high	50	few	married	yes	few	normal	no
6	2	middle	40	more	married	yes	more	obese	no
10	2	middle	55	more	married	no	more	obese	no
2	2	low	48	few	married	yes	few	obese	yes
26	2	high	37	more	married	yes	more	obese	no
9	2	Very low	58	more	married	yes	more	obese	no
29	3	high	35	more	unmarried	yes	few	obese	no
33	3	high	43	more	married	yes	few	obese	no
14	3	middle	35	more	married	yes	more	obese	no
25	3	middle	40	more	unmarried	yes	more	normal	yes
12	3	low	45	more	married	yes	more	obese	no
3	3	Very low	50	more	married	yes	more	obese	no
19	3	high	60	more	married	yes	more	obese	no
16	3	low	52	more	married	yes	more	obese	no
27	3	high	42	more	married	yes	more	obese	no
13	3	Very low	45	more	married	yes	more	obese	no
22	4	high	47	more	married	no	more	obese	yes
20	4	middle	30	more	married	yes	more	normal	no
24	4	middle	46	more	married	no	more	obese	no
21	4	middle	60	more	married	no	more	normal	no
1	4	middle	35	more	married	no	few	obese	no
17	4	low	40	more	unmarried	no	more	normal	no
4	4	Very low	47	more	unmarried	no	few	obese	no

4.1.3. The levels of Melatonin in the breast cancer patient and control groups according to the obesity

In this thesis, obesity was determined according to the body mass index (BMI), BMI is equal to the mass (Kg) / [height (m)] ^{2}. If the BMI range ≤ 18.5, it is considered underweight and when this range is in the 18.5 to 25, the person is considered normal but if the BMI value is 25 to 30, the person is considered overweight but if it is equal to 30 and above, this range is indicated obesity. Our results show that obesity can cause decrease melatonin levels. For this purpose, this level was determined according to the BMI.

The figure 4.7. shows that the relationship between the levels of melatonin, breast cancer patients who have normal and overweight regarding BMI. Our findings show the levels of this pineal hormone for those who had an obese, were significantly deceased compare to those who had a normal or overweight.

In the patient group, the maximum levels of melatonin at 2:00 was equal to (37.2 Pg/ml) in the sample (29) and this level was reached to a minimum to (20.8 Pg/ml) in the sample (9) while this level at 9:00, the maximum level was (15.7 Pg/ml) in the sample number (29) and was decreased to minimum value was (7.1 Pg/ml) in the sample number (9).

Our findings show that overweight samples have high levels of melatonin than those who are normal and obese. In the patient group, the levels of melatonin were significantly high in the samples (29, 26, 28, 14, 22, 33, 27, and 32) who have the BMI in range (25 – 30), these groups have higher levels than others. If noticed that they have not the same peaks, this may be related to their ages, stages of their disease, chemotherapy treatment, and sleep duration. The sample (29) had a high peak and the sample (4) had a low peak, this difference in peaks may be related to those factors above, the sample (29) was 35 years old, she was in stage 3 and had received few chemotherapy treatment, and had also slept more while the sample (4) was 47 years old, she was in stage 4, she had received more chemotherapy treatment, and was slept for few hours.

Overall, the results of this study show the melatonin levels change in normal, overweight, and obese patients, this level was slightly increased in a normal group (18.5 – 25 BMI) and was reached the maximum in an overweight group (25 – 30 BMI) but this level was significantly decreased in an obese group (30 BMI and above).

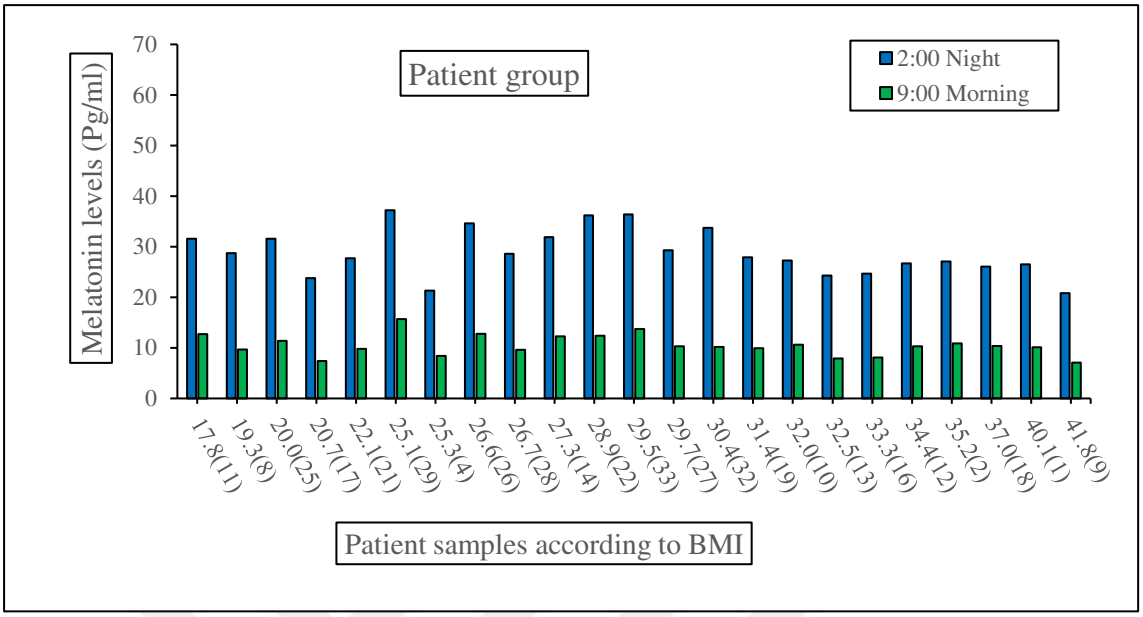


Figure 4.7. Melatonin levels in the patient group according to BMI

The figure 4.8. shows the maximum and minimum levels of melatonin in the control group In this group, the maximum levels of melatonin at 2:00 was equal to (65.9 Pg/ml) in the sample (22) and this level was reached to a minimum to (42.9 Pg/ml) in the sample (13) while this level at 9:00, the maximum level was (19.4 Pg/ml) in the sample (8) and was decreased to minimum value was (10.4 Pg/ml) in the sample (2).

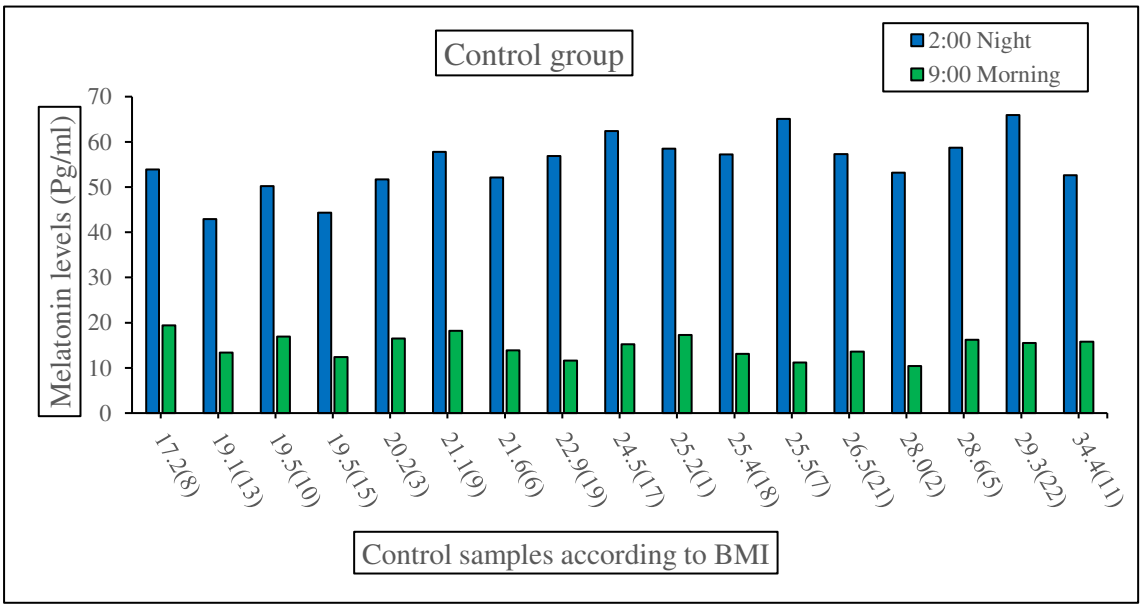


Figure 4.8. Melatonin levels in the control group according to BMI

4.1.4. The levels of melatonin in the breast cancer patient and control groups according to a Family history

Domestic experience is one of the most important factors which cause develop the risk of breast cancer especially those who have first-degree relatives for example parents and siblings. In this study, we tried to find the relationship between the levels of melatonin and domestic experience in patient and control groups but it is important to say that we did not find a link between these two parameters. In other words, this level was not dependent on the number of patients and controls who have had a family history or didn't and this level didn't change systematically in these groups but the important point found in this thesis was the levels of this hormone was significantly increased in the nighttime at 2:00 in the night and decreased in the daytime at 9:00 am. Another point is that all the control group had a family history because they were relatives with the patient group.

The figure 4.9 shows the maximum and minimum levels of melatonin in the breast cancer patient group, the melatonin levels at 2:00 were reached to the maximum. The top-level was equal to (38.6 Pg/ml) in the sample (30) and reached to minimum level was (20.8 Pg/ml) in the sample (9) while this level was significantly decreased at 9:00 compares to this level at 2:00. In the morning at 9:00, the maximum level was (14.8 Pg/ml) in the sample (30) and the minimum was equal to (7.1P g/ml) in the sample (9) this result has shown in

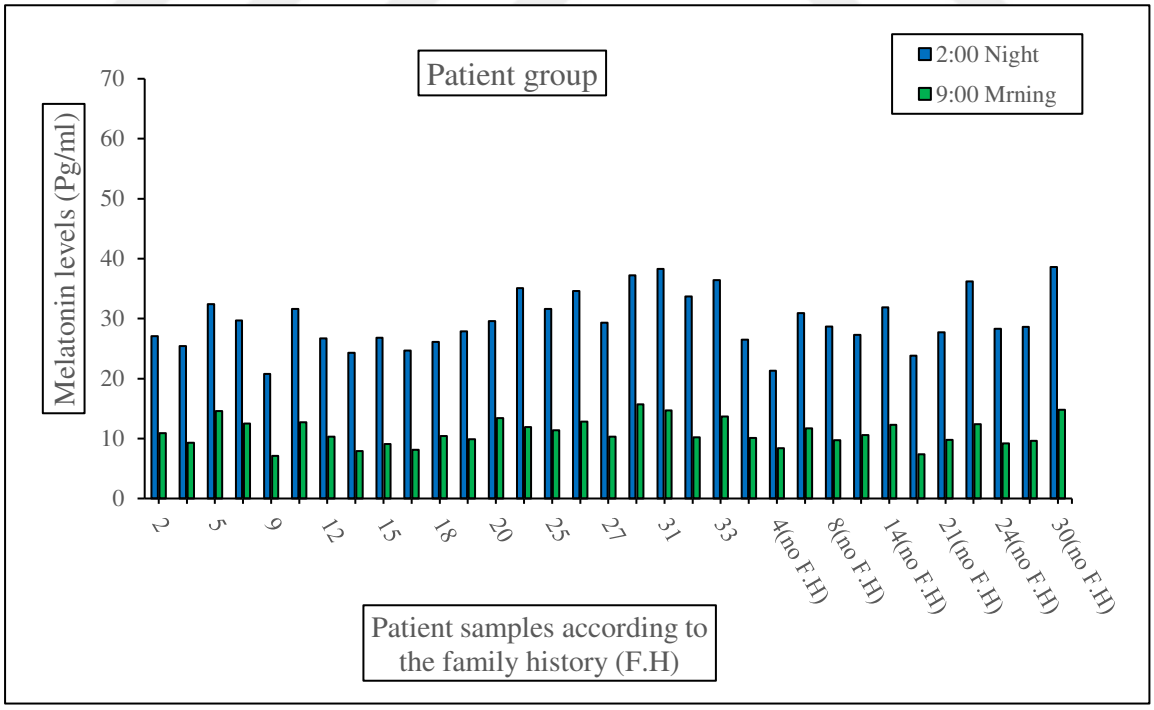


Figure 4.9. Melatonin levels in the patient group according to a Family history

4.2. The levels of total antioxidants in the breast cancer patient and control groups

Antioxidants have a positive result in body by protecting cells against free radicals. Total levels of antioxidants depend on the level of each oxidative stress and oxidants, both oxidative stress and oxidants will cause to decrease in the activity of total antioxidants, meaning that the total antioxidants levels will decrease by increasing the rate of both oxidative stress and oxidants. Melatonin acts as antioxidants, those factors that cause to decrease melatonin levels may cause to decrease the levels of total antioxidants at the same time.

The table 4.3 illustates the statistical calculation of mean, Std. deviation, Paired T-test, Std. Error, and P-value for serum total antioxidants have been done by SPSS Ststistical analysis.

Table 4.3. Paired T-test for the patient and control groups

Paired samples T-test for the patient group **Paired Samples Statistics**				
	Mean	N	Std. Deviation	Std. Error Mean
Pair 1 Night Morning	1.2446 0.7468	33 33	0.12184 0.36637	0.02121 0.06378
Paired Samples Correlations				
	N	Correlation	Sig.	
Pair 1 Night & Morning	33	0.412	0.017	
Paired samples T-test for the control group **Paired Samples Statistics**				
	Mean	N	Std. Deviation	Std. Error Mean
Pair 1 Night Morning	1.4603 1.0605	33 33	0.17392 0.23185	0.03028 0.04036
Paired Samples Correlations				
	N	Correlation	Sig.	
Pair 1 Night & Morning	33	0.546	0.001	

Figure 4.10. and Figure 4.11. show the levels of total antioxidants in breast cancer patients and the control group according to the number of samples respectively. In these figures, the blue color is represented the hour of sleeping at 2:00 in the night and the green color is represented the hour of awake 9:00 in the morning.

According to our results, the levels of total antioxidants were high at 2:00 at night and low at 9:00 in the morning in both the patient and control groups but this level more significantly higher in the control than the patient group.

The figure 4.10. shows the maximum and minimum levels of total antioxidants in the breast cancer patient group, the maximum level of total antioxidants at 2:00 at night was equal to (1.48 mmol/l) in the sample (25) and the minimum level was equal to (1.03 mmol/l) in the sample (22) as well as the maximum level at 9:00 in the morning was reached (1.3 mmol/l) in the sample (12) and the minimum level was (0.05 mmol/l) in the sample (8).

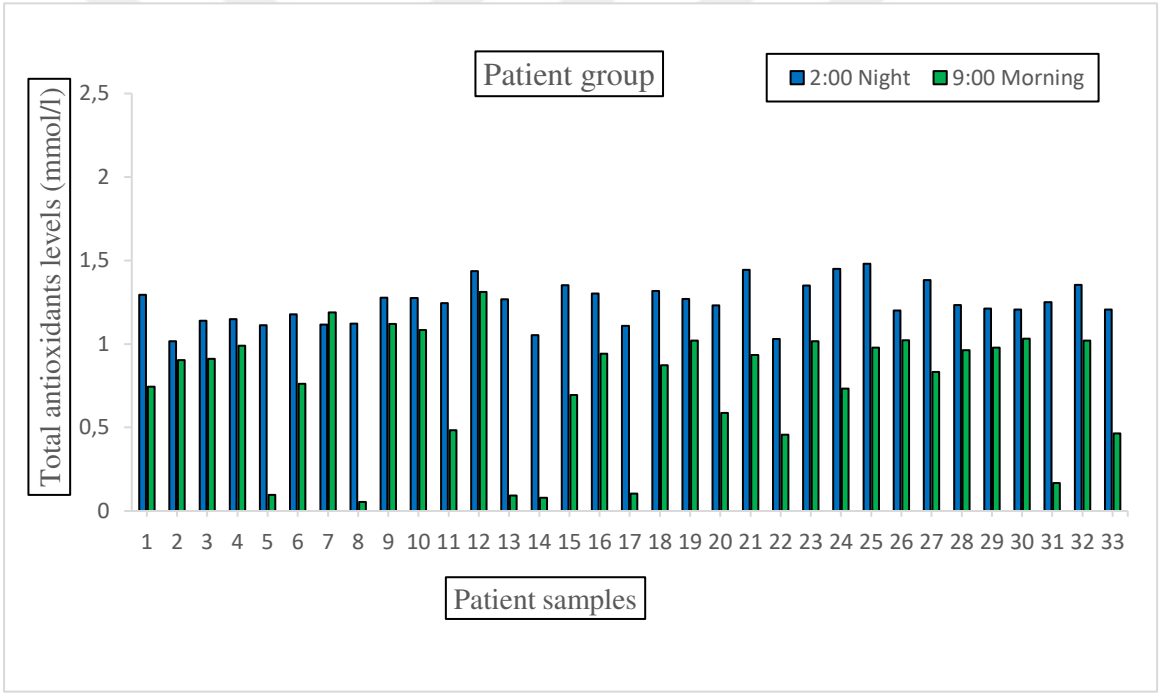


Figure 4.10. Total antioxidants levels in the patient group

The figure 4.11. shows the maximum and minimum levels of total antioxidants in the control group, the maximum level of total antioxidants at 2:00 at night was equal to (1.91mmol/l) in the sample (20) and the minimum level was (1.33 mmol/l) in the sample (15) as well as the maximum level at 9:00 am was reached (1.35 mmol/l) in the sample (10) and the minimum level was equal to (1.02mmol/l) in the sample (15).

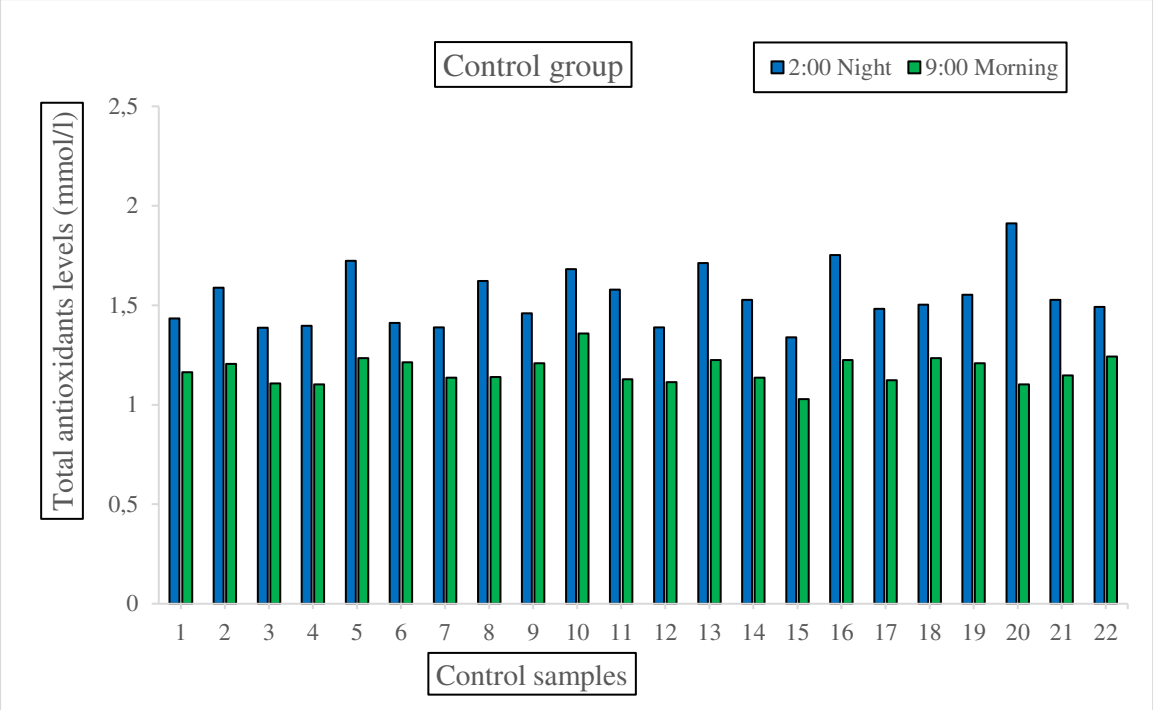


Figure 4.11. Total antioxidants levels in the control group

5. CONCLUSION

From the obtained result, it can be concluded that:

1- According to our results, the levels of melatonin were reached a maximum at 2:00 at night and after it started to decrease at 9:00 in the morning and became the minimum value at this time.

2- The results show that the levels of this hormone were changed in different ages and the levels were decreased in older compare to the younger.

3- Melatonin levels were changed at two different times (2:00 and 9:00) according to the chemotherapy treatment, the levels were low in those patients who had more received chemotherapy treatment compares to those who had received few.

4- Our findings illustrate that the stage of this disease is also very important that must be considered, the levels of this pineal hormone were significantly lower in those patients who were in high stages in their disease than those who were in low stages.

5- Obesity was another important factor that caused to decrease in the levels of the pineal hormone. This level in both patient and control groups was low among those who were obese and was high in control and overweight.

6- Family history was not affected on the levels of melatonin according to these results while those have significant effects on breast cancer.

7- The levels of antioxidants were reached to a maximum at 2:00 at night and after it started to decrease at 9:00 in the morning and reached the minimum value at this time.

8- Sleep duration was a directly affected to decrease or increase the levels of both melatonin and total antioxidants in humans.

RECOMMENDATIONS

In the future work, this work will be done in another way; this will determine the effect of melatonin and total antioxidant capacity in both group (Patients with breast cancer and Healthy group) at the same time (i.e. at 2:00 in the night and 9:00 in the morning), and compare these groups between each other.

REFERENCES

[1] Lee, M.S., Pittler, M.H., and Ernst, E. (2007). Is Tai Chi an effective adjunct in cancer care? A systematic review of controlled clinical trials, *Supportive Care in Cancer*, C Vol. 15 (6), pp: 597-601.

[2] Siegel, R.L., Miller, K.D., and Jemal, A. (2020). Cancer statistics, 2020, *CA: a cancer journal for clinicians*, C Vol. 70 (1), pp: 7-30.

[3] Hansen, J. (2006). Risk of breast cancer after night-and shift work: current evidence and ongoing studies in Denmark, *Cancer Causes & Control*, C Vol. 17 (4), pp: 531-537.

[4] Mustafa, M., Nornazirah, A., Salih, F., Illzam, E., Suleiman, M., and Sharifa, A. (2016). Breast cancer: detection markers, prognosis, and prevention, *IOSR Journal of Dental and Medical Sciences*, C Vol. 15 (08), pp: 73-80.

[5] Bombardieri, E., Bonadonna, G., and Gianni, L., (2007). *Breast cancer: nuclear medicine in diagnosis and therapeutic options*, Springer Science & Business Media,

[6] Hill, S.M., Belancio, V.P., Dauchy, R.T., Xiang, S., Brimer, S., Mao, L., Hauch, A., Lundberg, P.W., Summers, W., and Yuan, L. (2015). Melatonin: an inhibitor of breast cancer, *Endocrine-related cancer*, C Vol. 22 (3), pp: R183-R204.

[7] Talebianpoor, M., Khodadoust, S., Rozbehi, A., Toori, M.A., Zoladl, M., Ghaedi, M., Mohammadi, R., and Hosseinzadeh, A. (2014). Application of optimized dispersive liquid–liquid microextraction for determination of melatonin by HPLC–UV in plasma samples, *Journal of Chromatography B*, C Vol. 960 1-7.

[8] Pandi-Perumal, S.R., Srinivasan, V., Maestroni, G., Cardinali, D., Poeggeler, B., and Hardeland, R. (2006). Melatonin: nature's most versatile biological signal?, *The FEBS journal*, C Vol. 273 (13), pp: 2813-2838.

[9] Reiter, R.J., Calvo, J.R., Karbownik, M., Qi, W., and Tan, D.X. (2000). Melatonin and its relation to the immune system and inflammation, *Annals of the New York Academy of Sciences*, C Vol. 917 (1), pp: 376-386.

[10] Slominski, R.M., Reiter, R.J., Schlabritz-Loutsevitch, N., Ostrom, R.S., and Slominski, A.T. (2012). Melatonin membrane receptors in peripheral tissues: distribution and functions, *Molecular and cellular endocrinology*, C Vol. 351 (2), pp: 152-166.

[11] Arendt, J. (2005). Melatonin: characteristics, concerns, and prospects, *Journal of Biological Rhythms*, C Vol. 20 (4), pp: 291-303.

[12] Kabel, A.M. (2014). Free radicals and antioxidants: role of enzymes and nutrition, *World Journal of Nutrition and Health*, C Vol. 2 (3), pp: 35-38.

[13] Ríos-Arrabal, S., Artacho-Cordón, F., León, J., Román-Marinetto, E., del Mar Salinas-Asensio, M., Calvente, I., and Núñez, M.I. (2013). Involvement of free radicals in breast cancer, *Springerplus*, C Vol. 2 (1), pp: 404.

[14] Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y., and De, B. (2010). Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect, *International journal of pharmaceutical sciences review and research*, C Vol. 3 (1), pp: 91-100.

[15] Kampa, M., Nistikaki, A., Tsaousis, V., Maliaraki, N., Notas, G., and Castanas, E. (2002). A new automated method for the determination of the Total Antioxidant Capacity (TAC) of human plasma, based on the crocin bleaching assay, *BMC clinical pathology*, C Vol. 2 (1), pp: 1-16.

[16] Mahmood, I.H., Abdullah, K.S., and Abdullah, M.S. (2009). Total antioxidant status in women with breast cancer, *Pak J Med Sci*, C Vol. 25 (4), pp: 609-12.

[17] Stanner, S., Hughes, J., Kelly, C., and Buttriss, J. (2004). A review of the epidemiological evidence for the 'antioxidant hypothesis', *Public health nutrition*, C Vol. 7 (3), pp: 407-422.

[18] Pantavos, A., Ruiter, R., Feskens, E.F., de Keyser, C.E., Hofman, A., Stricker, B.H., Franco, O.H., and Kiefte-de Jong, J.C. (2015). Total dietary antioxidant capacity, individual antioxidant intake and breast cancer risk: the R otterdam study, *International journal of cancer*, C Vol. 136 (9), pp: 2178-2186.

[19] Reis-Filho, J. and Lakhani, S.R. (2008). Breast cancer special types: why bother?, *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, C Vol. 216 (4), pp: 394-398.

[20] Jasser, S.A., Blask, D.E., and Brainard, G.C. (2006). Light during darkness and cancer: relationships in circadian photoreception and tumor biology, *Cancer Causes & Control*, C Vol. 17 (4), pp: 515-523.

[21] Deshpande, T.M., Pandey, A., and Shyama, S. (2017). Breast cancer and etiology, Vol.

[22] Nargis, H., Nawaz, H., Ditta, A., Mahmood, T., Majeed, M., Rashid, N., Muddassar, M., Bhatti, H., Saleem, M., and Jilani, K. (2019). Raman spectroscopy of blood plasma samples from breast cancer patients at different stages, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, C Vol. 222 117210.

[23] Makki, J. (2015). Diversity of breast carcinoma: histological subtypes and clinical relevance, *Clinical Medicine Insights: Pathology*, C Vol. 8 CPath. S31563.

[24] Weigelt, B., Geyer, F.C., and Reis-Filho, J.S. (2010). Histological types of breast cancer: how special are they?, *Molecular oncology*, C Vol. 4 (3), pp: 192-208.

[25] Marshall, L.M., Hunter, D.J., Connolly, J.L., Schnitt, S.J., Byrne, C., London, S.J., and Colditz, G.A. (1997). Risk of breast cancer associated with atypical hyperplasia of lobular and ductal types, *Cancer Epidemiology and Prevention Biomarkers*, C Vol. 6 (5), pp: 297-301.

[26] Weigelt, B. and Reis-Filho, J.S. (2009). Histological and molecular types of breast cancer: is there a unifying taxonomy?, *Nature Reviews Clinical Oncology*, C Vol. 6 (12), pp: 718.

[27] Ellis, P., Schnitt, S., Sastre-Garau, X., Bussolati, G., Tavassoli, F., Eusebi, V., and Cornelisse, C. (2003). WHO classification of tumours. pathology and genetics of tumours of the breast and female genital organs, *Pathology and genetics of tumors of the breast and female genital organs. Lyon Press, Lyon*, C Vol.

[28] Rakha, E., Putti, T., Abd El-Rehim, D., Paish, C., Green, A., Powe, D., Lee, A., Robertson, J., and Ellis, I. (2006). Morphological and immunophenotypic analysis of breast carcinomas with basal and myoepithelial differentiation, *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, C Vol. 208 (4), pp: 495-506.

[29] Rakha, E.A., Reis-Filho, J.S., and Ellis, I.O. (2008). Basal-like breast cancer: a critical review, *Journal of clinical oncology*, C Vol. 26 (15), pp: 2568-2581.

[30] Kelsey, J.L., Gammon, M.D., and John, E.M. (1993). Reproductive factors and breast cancer, *Epidemiologic reviews*, C Vol. 15 (1), pp: 36.

[31] Sharifian, A., Farahani, S., Pasalar, P., Gharavi, M., and Aminian, O. (2005). Shift work as an oxidative stressor, *Journal of circadian rhythms*, C Vol. 3 (1), pp: 15.

[32] Lie, J.-A.S., Roessink, J., and Kjaerheim, K. (2006). Breast cancer and night work among Norwegian nurses, *Cancer Causes & Control*, C Vol. 17 (1), pp: 39-44.

[33] Feng, J.-F., Lu, L., Zeng, P., Yang, Y.-H., Luo, J., Yang, Y.-W., and Wang, D. (2012). Serum total oxidant/antioxidant status and trace element levels in breast cancer patients, *International journal of clinical oncology*, C Vol. 17 (6), pp: 575-583.

[34] Verkasalo, P., Pukkala, E., Stevens, R., Ojamo, M., and Rudanko, S.-L. (1999). Inverse association between breast cancer incidence and degree of visual impairment in Finland, *British journal of cancer*, C Vol. 80 (9), pp: 1459-1460.

[35] Lee, H.-E., Lee, J., Jang, T.-W., Kim, I.-A., Park, J., and Song, J. (2018). The relationship between night work and breast cancer, *Annals of occupational and environmental medicine*, C Vol. 30 (1), pp: 11.

[36] Cos, S., González, A., Martínez-Campa, C., Mediavilla, M.D., Alonso-González, C., and Sánchez-Barceló, E.J. (2006). Estrogen-signaling pathway: a link between breast cancer and melatonin oncostatic actions, *Cancer detection and prevention*, C Vol. 30 (2), pp: 118-128.

[37] McELROY, J.A., Newcomb, P.A., TITUS-ERNSTOFF, L., TRENTHAM-DIETZ, A., Hampton, J.M., and Egan, K.M. (2006). Duration of sleep and breast cancer risk in a large population-based case–control study, *Journal of sleep research*, C Vol. 15 (3), pp: 241-249.

[38] Schernhammer, E.S. and Hankinson, S.E. (2005). Urinary melatonin levels and breast cancer risk, *Journal of the National Cancer Institute*, C Vol. 97 (14), pp: 1084-1087.

[39] Viswanathan, A.N. and Schernhammer, E.S. (2009). Circulating melatonin and the risk of breast and endometrial cancer in women, *Cancer letters*, C Vol. 281 (1), pp: 1-7.

[40] Korkmaz, A., Sanchez-Barcelo, E.J., Tan, D.-X., and Reiter, R.J. (2009). Role of melatonin in the epigenetic regulation of breast cancer, *Breast cancer research and treatment*, C Vol. 115 (1), pp: 13-27.

[41] Richter, K., Acker, J., Kamcev, N., Bajraktarov, S., Piehl, A., and Niklewski, G. (2011). Recommendations for the prevention of breast cancer in shift workers, *EPMA Journal*, C Vol. 2 (4), pp: 351-356.

[42] SOINI, I. (1977). Risk factors of breast cancer in Finland, *International Journal of Epidemiology*, C Vol. 6 (4), pp: 365-373.

[43] Helmrich, S.P., SHAPIRO, S., ROSENBERG, L., KAUFMAN, D.W., SLONE, D., BAIN, C., MIETTINEN, O.S., STOLLEY, P.D., ROSENSHEIN, N.B., and KNAPP, R.C. (1983). Risk factors for breast cancer, *American journal of epidemiology*, C Vol. 117 (1), pp: 35-45.

[44] McTIERNAN, A. and THOMAS, D.B. (1986). Evidence for a protective effect of lactation on risk of breast cancer in young women: results from a case-control study, *American journal of epidemiology*, C Vol. 124 (3), pp: 353-358.
[45] BRISSON, J., VERREAULT, R., MORRISON, A.S., TENNINA, S., and MEYER, F. (1989). Diet, mammographic features of breast tissue, and breast cancer risk, *American journal of epidemiology*, C Vol. 130 (1), pp: 14-24.
[46] Gerber, B., Müller, H., Reimer, T., Krause, A., and Friese, K. (2003). Nutrition and lifestyle factors on the risk of developing breast cancer, *Breast cancer research and treatment*, C Vol. 79 (2), pp: 265-276.
[47] Pérez-Solis, M.A., Maya-Nuñez, G., Casas-González, P., Olivares, A., and Aguilar-Rojas, A. (2016). Effects of the lifestyle habits in breast cancer transcriptional regulation, *Cancer cell international*, C Vol. 16 (1), pp: 1-11.
[48] Slattery, M.L. and Kerber, R.A. (1993). A comprehensive evaluation of family history and breast cancer risk: the Utah Population Database, *Jama*, C Vol. 270 (13), pp: 1563-1568.
[49] Key, T.J., Verkasalo, P.K., and Banks, E. (2001). Epidemiology of breast cancer, *The lancet oncology*, C Vol. 2 (3), pp: 133-140.
[50] Favero, A., Parpinel, M., and Franceschi, S. (1998). Diet and risk of breast cancer: major findings from an Italian case-control study, *Biomedicine & pharmacotherapy*, C Vol. 52 (3), pp: 109-115.
[51] Seiler, A., Chen, M.A., Brown, R.L., and Fagundes, C.P. (2018). Obesity, dietary factors, nutrition, and breast cancer risk, *Current breast cancer reports*, C Vol. 10 (1), pp: 14-27.
[52] Moysich, K.B., Ambrosone, C.B., Vena, J.E., Shields, P.G., Mendola, P., Kostyniak, P., Greizerstein, H., Graham, S., Marshall, J.R., and Schisterman, E.F. (1998). Environmental organochlorine exposure and postmenopausal breast cancer risk, *Cancer Epidemiology and Prevention Biomarkers*, C Vol. 7 (3), pp: 181-188.
[53] Chowdhury, I., Sengupta, A., and Maitra, S.K. (2008). Melatonin: fifty years of scientific journey from the discovery in bovine pineal gland to delineation of functions in human, Vol.
[54] Hevia, D., Mayo, J., Quiros, I., Gomez-Cordoves, C., and Sainz, R. (2010). Monitoring intracellular melatonin levels in human prostate normal and cancer cells by HPLC, *Analytical and bioanalytical chemistry*, C Vol. 397 (3), pp: 1235-1244.
[55] Wu, A.H., Wang, R., Koh, W.-P., Stanczyk, F.Z., Lee, H.-P., and Yu, M.C. (2008). Sleep duration, melatonin and breast cancer among Chinese women in Singapore, *Carcinogenesis*, C Vol. 29 (6), pp: 1244-1248.
[56] Yu, H.-S. and Reiter, R.J., (2020). *Melatonin: biosynthesis, physiological effects, and clinical applications*, cRC Press,
[57] Grivas, T.B. and Savvidou, O.D. (2007). Melatonin the" light of night" in human biology and adolescent idiopathic scoliosis, *Scoliosis*, C Vol. 2 (1), pp: 6.
[58] Blakeman, V., Williams, J.L., Meng, Q.-J., and Streuli, C.H. (2016). Circadian clocks and breast cancer, *Breast Cancer Research*, C Vol. 18 (1), pp: 89.
[59] WANG, J.z. and WANG, Z.f. (2006). Role of melatonin in Alzheimer-like neurodegeneration 1, *Acta Pharmacologica Sinica*, C Vol. 27 (1), pp: 41-49.
[60] Dawson, D. and Armstrong, S.M. (1996). Chronobiotics—drugs that shift rhythms, *Pharmacology & therapeutics*, C Vol. 69 (1), pp: 15-36.
[61] Posadzki, P.P., Bajpai, R., Kyaw, B.M., Roberts, N.J., Brzezinski, A., Christopoulos, G.I., Divakar, U., Bajpai, S., Soljak, M., and Dunleavy, G. (2018). Melatonin and health: an umbrella review of health outcomes and biological mechanisms of action, *BMC medicine*, C Vol. 16 (1), pp: 18.
[62] Srinivasan, V., Spence, D., Pandi-Perumal, S., Trakht, I., Esquifino, A., Cardinali, D., and Maestroni, G. (2008). Melatonin, environmental light, and breast cancer, *Breast cancer research and treatment*, C Vol. 108 (3), pp: 339-350.
[63] Grant, S.G., Melan, M.A., Latimer, J.J., and Witt-Enderby, P.A. (2009). Melatonin and breast cancer: cellular mechanisms, clinical studies and future perspectives, *Expert reviews in molecular medicine*, C Vol. 11 e5.
[64] Tyagi, E., Agrawal, R., Nath, C., and Shukla, R. (2010). Effect of melatonin on neuroinflammation and acetylcholinesterase activity induced by LPS in rat brain, *European Journal of Pharmacology*, C Vol. 640 (1-3), pp: 206-210.
[65] Liu, L., Wang, T., Yang, X., Xu, C., Liao, Z., Wang, X., Su, D., Li, Y., Zhou, H., and Qiu, X. (2019). MTNR1B loss promotes chordoma recurrence by abrogating melatonin-mediated β-catenin signaling repression, *Journal of pineal research*, C Vol. 67 (2), pp: e12588.
[66] Sugden, D., Davidson, K., Hough, K.A., and Teh, M.T. (2004). Melatonin, melatonin receptors and melanophores: a moving story, *Pigment Cell Research*, C Vol. 17 (5), pp: 454-460.

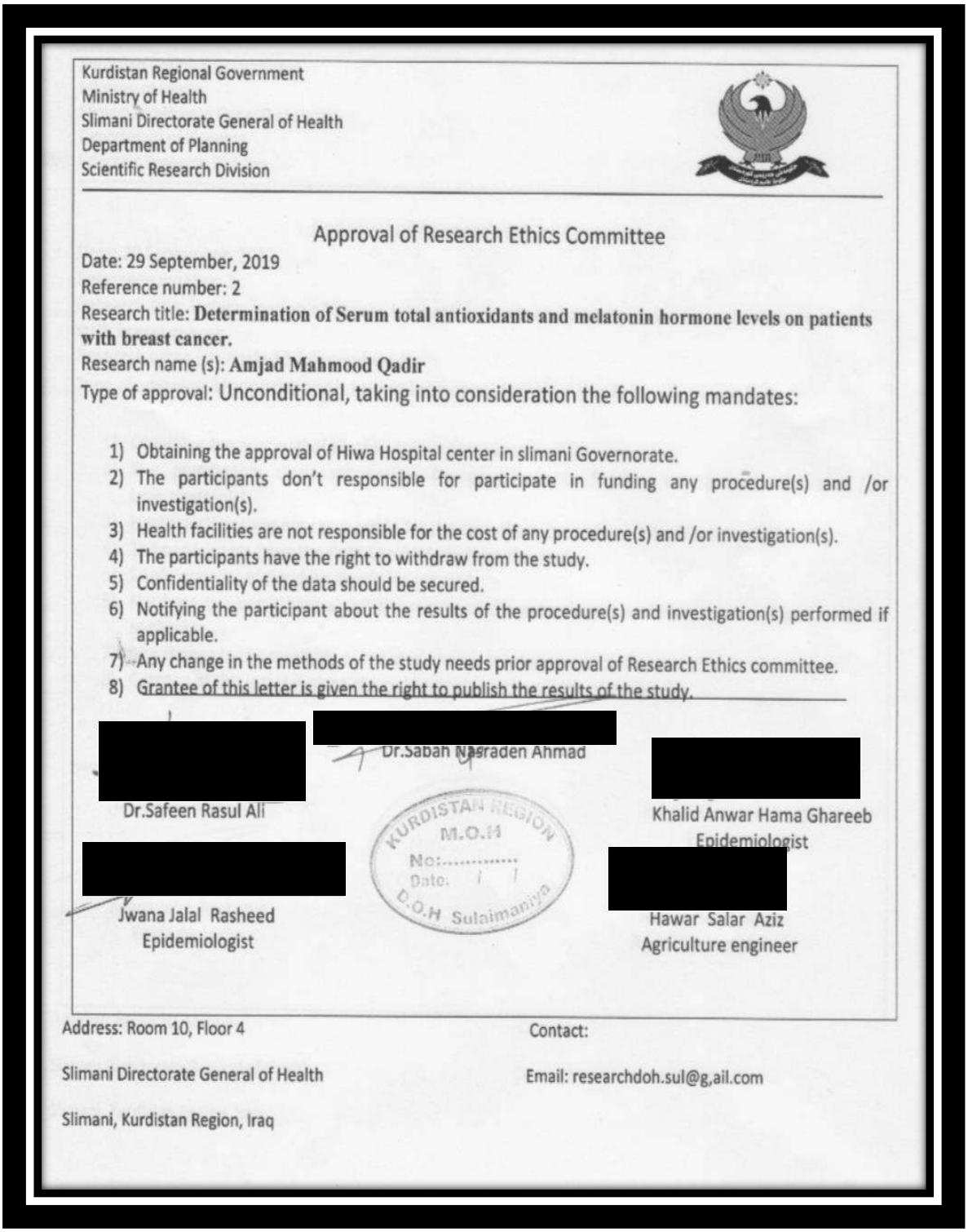
[67] Zhao, D., Yu, Y., Shen, Y., Liu, Q., Zhao, Z., Sharma, R., and Reiter, R.J. (2019). Melatonin synthesis and function: evolutionary history in animals and plants, *Frontiers in endocrinology*, C Vol. 10 249.
[68] Tan, D., Reiter, R.J., Manchester, L.C., Yan, M., El-Sawi, M., Sainz, R.M., Mayo, J.C., Kohen, R., Allegra, M., and Hardeland, R. (2002). Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger, *Current Topics in Medicinal Chemistry*, C Vol. 2 (2), pp: 181-197.
[69] Reiter, R.J., Melchiorri, D., Sewerynek, E., Poeggeler, B., Barlow-Walden, L., Chuang, J., Ortiz, G.G., and AcuñaCastroviejo, D. (1995). A review of the evidence supporting melatonin's role as an antioxidant, *Journal of Pineal Research*, C Vol. 18 (1), pp: 1-11.
[70] Bonnefont-Rousselot, D. and Collin, F. (2010). Melatonin: action as antioxidant and potential applications in human disease and aging, *Toxicology*, C Vol. 278 (1), pp: 55-67.
[71] Najafi, M., Shirazi, A., Motevaseli, E., Rezaeyan, A., Salajegheh, A., and Rezapoor, S. (2017). Melatonin as an anti-inflammatory agent in radiotherapy, *Inflammopharmacology*, C Vol. 25 (4), pp: 403-413.
[72] Agarwal, A., Gupta, S., and Sharma, R. (2005). Oxidative stress and its implications in female infertility–a clinician's perspective, *Reproductive biomedicine online*, C Vol. 11 (5), pp: 641-650.
[73] Bhatia, A. and Manda, K. (2004). Study on pre-treatment of melatonin against radiation-induced oxidative stress in mice, *Environmental Toxicology and Pharmacology*, C Vol. 18 (1), pp: 13-20.
[74] Proietti, S., Cucina, A., Reiter, R.J., and Bizzarri, M. (2013). Molecular mechanisms of melatonin's inhibitory actions on breast cancers, *Cellular and molecular life sciences*, C Vol. 70 (12), pp: 2139-2157.
[75] Mirick, D.K. and Davis, S. (2008). Melatonin as a biomarker of circadian dysregulation, *Cancer Epidemiology and Prevention Biomarkers*, C Vol. 17 (12), pp: 3306-3313.
[76] Bespyatykh, A.Y., Burlakova, O., and Golichenkov, V. (2011). Melatonin as an antioxidant: The main functions and properties, *Biology Bulletin Reviews*, C Vol. 1 (2), pp: 143.
[77] Tomás-Zapico, C. and Coto-Montes, A. (2007). Melatonin as antioxidant under pathological processes, *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*, C Vol. 1 (1), pp: 63-82.
[78] Kotler, M., Rodríguez, C., Sáinz, R.M., Antolin, I., and Menéndez-Peláez, A. (1998). Melatonin increases gene expression for antioxidant enzymes in rat brain cortex, *Journal of Pineal Research*, C Vol. 24 (2), pp: 83-89.
[79] Eck-Enriquez, K., TAG, M.E., Spriggs, L.L., and Hill, S.M. (2000). Pathways through which a regimen of melatonin and retinoic acid induces apoptosis in MCF-7 human breast cancer cells, *Breast Cancer Research and Treatment*, C Vol. 61 (3), pp: 229-239.
[80] Di Bella, G., Mascia, F., Gualano, L., and Di Bella, L. (2013). Melatonin anticancer effects, *International journal of molecular sciences*, C Vol. 14 (2), pp: 2410-2430.
[81] Anwar, M.M., Mahfouz, H.A., and Sayed, A.S. (1998). Potential protective effects of melatonin on bone marrow of rats exposed to cytotoxic drugs, *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, C Vol. 119 (2), pp: 493-501.
[82] Mauriz, J.L., Collado, P.S., Veneroso, C., Reiter, R.J., and González-Gallego, J. (2013). A review of the molecular aspects of melatonin's anti-inflammatory actions: recent insights and new perspectives, *Journal of pineal research*, C Vol. 54 (1), pp: 1-14.
[83] Favero, G., Franceschetti, L., Bonomini, F., Rodella, L.F., and Rezzani, R. (2017). Melatonin as an anti-inflammatory agent modulating inflammasome activation, *International Journal of Endocrinology*, C Vol. 2017.
[84] Carrillo-Vico, A., Reiter, R.J., Lardone, P.J., Herrera, J.L., Fernández-Montesinos, R., Guerrero, J.M., and Pozo, D. (2006). The modulatory role of melatonin on immune responsiveness, *Current opinion in investigational drugs*, C Vol. 7 (5), pp: 423.
[85] Guerrero, J.M. and Reiter, R.J. (2002). Melatonin-immune system relationships, *Current topics in medicinal chemistry*, C Vol. 2 (2), pp: 167-179.
[86] Liebmann, P.M., Wölfler, A., Felsner, P., Hofer, D., and Schauenstein, K. (1997). Melatonin and the immune system, *International archives of allergy and immunology*, C Vol. 112 (3), pp: 203-211.
[87] Smythe, G. and Lazarus, L. (1973). Growth hormone regulation by melatonin and serotonin, *Nature*, C Vol. 244 (5413), pp: 230-231.
[88] Valcavi, R., Zini, M., Maestroni, G.J., Conti, A., and Portioli, I. (1993). Melatonin stimulates growth hormone secretion through pathways other than the growth hormone-releasing hormone, *Clinical endocrinology*, C Vol. 39 (2), pp: 193-199.

[89] La Fleur, S.E., Kalsbeek, A., Wortel, J., Van Der Vliet, J., and Buijs, R. (2001). Role for the pineal and melatonin in glucose homeostasis: pinealectomy increases night-time glucose concentrations, *Journal of neuroendocrinology*, C Vol. 13 (12), pp: 1025-1032.
[90] la Fleur, S.E., Kalsbeek, A., Wortel, J., Fekkes, M.L., and Buijs, R.M. (2001). A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus, *Diabetes*, C Vol. 50 (6), pp: 1237-1243.
[91] Korkmaz, A., Topal, T., Tan, D.-X., and Reiter, R.J. (2009). Role of melatonin in metabolic regulation, *Reviews in Endocrine and Metabolic Disorders*, C Vol. 10 (4), pp: 261-270.
[92] Cos, S. and Blask, D.E. (1994). Melatonin modulates growth factor activity in MCF-7 human breast cancer cells, *Journal of pineal research*, C Vol. 17 (1), pp: 25-32.
[93] Lissoni, P., Barni, S., Tancini, G., Fossati, V., and Frigerio, F. (1994). Pineal-opioid system interactions in the control of immunoinflammatory responses, *Annals of the New York Academy of Sciences*, C Vol. 741 191-196.
[94] Travis, R.C., Allen, D.S., Fentiman, I.S., and Key, T.J. (2004). Melatonin and breast cancer: a prospective study, *Journal of the National Cancer Institute*, C Vol. 96 (6), pp: 475-482.
[95] Nagata, C., Nagao, Y., Yamamoto, S., Shibuya, C., Kashiki, Y., and Shimizu, H. (2008). Light exposure at night, urinary 6-sulfatoxymelatonin, and serum estrogens and androgens in postmenopausal Japanese women, *Cancer Epidemiology and Prevention Biomarkers*, C Vol. 17 (6), pp: 1418-1423.
[96] Blaicher, W., Speck, E., Imhof, M.H., Gruber, D.M., Schneeberger, C., Sator, M.O., and Huber, J.C. (2000). Melatonin in postmenopausal females, *Archives of Gynecology and Obstetrics*, C Vol. 263 (3), pp: 116-118.
[97] Lewy, A.J., Wehr, T.A., Goodwin, F.K., Newsome, D.A., and Markey, S. (1980). Light suppresses melatonin secretion in humans, *Science*, C Vol. 210 (4475), pp: 1267-1269.
[98] Stevens, R.G. and Davis, S. (1996). The melatonin hypothesis: electric power and breast cancer, *Environmental health perspectives*, C Vol. 104 (suppl 1), pp: 135-140.
[99] Benot, S., Gobema, R., Reiter, R.J., Garcia-Mauriño, S., Osuna, C., and Guerrero, J.M. (1999). Physiological levels of melatonin contribute to the antioxidant capacity of human serum, *Journal of Pineal Research*, C Vol. 27 (1), pp: 59-64.
[100] Rohr, U.D. and Herold, J. (2002). Melatonin deficiencies in women, *Maturitas*, C Vol. 41 85-104.
[101] Brown, G.M., Young, S.N., Gauthier, S., Tsui, H., and Grota, L.J. (1979). Melatonin in human cerebrospinal fluid in daytime; its origin and variation with age, *Life Sciences*, C Vol. 25 (11), pp: 929-936.
[102] Ferrari, E., Fioravanti, M., Magri, F., and Solerte, S. (2000). Variability of interactions between neuroendocrine and immunological functions in physiological aging and dementia of the Alzheimer's type, *Annals of the New York Academy of Sciences*, C Vol. 917 (1), pp: 582-596.
[103] Commentz, J. and Helmke, K. (1995). Precocious puberty and decreased melatonin secretion due to a hypothalamic hamartoma, *Hormone Research in Paediatrics*, C Vol. 44 (6), pp: 271-275.
[104] Peschke, E., Stumpf, I., Bazwinsky, I., Litvak, L., Dralle, H., and Mühlbauer, E. (2007). Melatonin and type 2 diabetes–a possible link?, *Journal of Pineal Research*, C Vol. 42 (4), pp: 350-358.
[105] Leproult, R., Copinschi, G., Buxton, O., and Van Cauter, E. (1997). Sleep loss results in an elevation of cortisol levels the next evening, *Sleep*, C Vol. 20 (10), pp: 865-870.
[106] DeSantis, C., Jemal, A., Ward, E., and Thun, M.J. (2008). Temporal trends in breast cancer mortality by state and race, *Cancer Causes and Control*, C Vol. 19 (5), pp: 537-545.
[107] Mayo, J.C., Sainz, R.M., Tan, D.-X., Antolín, I., Rodríguez, C., and Reiter, R.J. (2005). Melatonin and Parkinson's disease, *Endocrine*, C Vol. 27 (2), pp: 169-178.
[108] Morera-Fumero, A.L., Diaz-Mesa, E., Abreu-Gonzalez, P., Fernandez-Lopez, L., and Guillen-Pino, F. (2017). A three-month longitudinal study of changes in day/night serum total antioxidant capacity in paranoid schizophrenia, *PloS One*, C Vol. 12 (12), pp: e0189348.
[109] Psotová, J., Zahálková, J., Hrbac, J., Simanek, V., and Bartek, J. (2001). Determination of total antioxidant capacity in plasma by cyclic voltammetry. Two case reports, *Biomedical Papers-Palacky University in Olomouc*, C Vol. 145 (2), pp: 81-83.
[110] Erel, O. (2004). A novel automated method to measure total antioxidant response against potent free radical reactions, *Clinical Biochemistry*, C Vol. 37 (2), pp: 112-119.
[111] Hecht, F., Pessoa, C.F., Gentile, L.B., Rosenthal, D., Carvalho, D.P., and Fortunato, R.S. (2016). The role of oxidative stress on breast cancer development and therapy, *Tumor biology*, C Vol. 37 (4), pp: 4281-4291.
[112] Gligorovski, S., Strekowski, R., Barbati, S., and Vione, D. (2015). Environmental implications of hydroxyl radicals (• OH), *Chemical Reviews*, C Vol. 115 (24), pp: 13051-13092.

[113] Lipinski, B. (2011). Hydroxyl radical and its scavengers in health and disease, *Oxidative Medicine and Cellular Longevity*, C Vol. 2011.
[114] Whitehead, T., Thorpe, G., and Maxwell, S. (1992). Enhanced chemiluminescent assay for antioxidant capacity in biological fluids, *Analytica Chimica Acta*, C Vol. 266 (2), pp: 265-277.
[115] Buico, A., Cassino, C., Ravera, M., Betta, P.-G., and Osella, D. (2009). Oxidative stress and total antioxidant capacity in human plasma, *Redox Report*, C Vol. 14 (3), pp: 125-131.
[116] Erten Şener, D., Gönenç, A., Akıncı, M., and Torun, M. (2007). Lipid peroxidation and total antioxidant status in patients with breast cancer, *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease*, C Vol. 25 (4), pp: 377-382.
[117] Kiokias, S. and Gordon, M. (2003). Dietary supplementation with a natural carotenoid mixture decreases oxidative stress, *European journal of clinical nutrition*, C Vol. 57 (9), pp: 1135-1140.
[118] Dan, Y. (2008). Biological functions of antioxidants in plant transformation, *In Vitro Cellular & Developmental Biology-Plant*, C Vol. 44 (3), pp: 149-161.
[119] Ambrosone, C.B. (2000). Oxidants and antioxidants in breast cancer, *Antioxidants & redox signaling*, C Vol. 2 (4), pp: 903-917.
[120] Pan, S.Y., Zhou, J., Gibbons, L., Morrison, H., Wen, S.W., and Group, C.C.R.E.R. (2011). Antioxidants and breast cancer risk-a population-based case-control study in Canada, *Bmc Cancer*, C Vol. 11 (1), pp: 372.
[121] Martysiak-Żurowska, D. and Wenta, W. (2012). A comparison of ABTS and DPPH methods for assessing the total antioxidant capacity of human milk, *Acta Scientiarum Polonorum Technologia Alimentaria*, C Vol. 11 (1), pp: 83-89.
[122] Pandhair, V. and Sekhon, B. (2006). Reactive oxygen species and antioxidants in plants: an overview, *Journal of plant Biochemistry and Biotechnology*, C Vol. 15 (2), pp: 71-78.
[123] Pietta, P.-G. (2000). Flavonoids as antioxidants, *Journal of natural products*, C Vol. 63 (7), pp: 1035-1042.
[124] Kaur, C. and Kapoor, H.C. (2001). Antioxidants in fruits and vegetables–the millennium's health, *International journal of food science & technology*, C Vol. 36 (7), pp: 703-725.
[125] Szeto, Y.T., Tomlinson, B., and Benzie, I.F. (2002). Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation, *British journal of nutrition*, C Vol. 87 (1), pp: 55-59.
[126] Noguchi, N., Watanabe, A., and Shi, H. (2000). Diverse functions of antioxidants, *Free radical research*, C Vol. 33 (6), pp: 809-817.
[127] Jacob, R.A. (1995). The integrated antioxidant system, *Nutrition research*, C Vol. 15 (5), pp: 755-766.
[128] Sen, S. and Chakraborty, R., *The role of antioxidants in human health*, in *Oxidative stress: diagnostics, prevention, and therapy*2011, ACS Publications. p. 1-37.
[129] De la Fuente, M. (2002). Effects of antioxidants on immune system ageing, *European journal of clinical nutrition*, C Vol. 56 (3), pp: S5-S8.
[130] Yadav, A., Kumari, R., Yadav, A., Mishra, J., Srivatva, S., and Prabha, S. (2016). Antioxidants and its functions in human body-A Review, *Res. Environ. Life Sci*, C Vol. 9 (11), pp: 1328-1331.
[131] Foyer, C.H. and Shigeoka, S. (2011). Understanding oxidative stress and antioxidant functions to enhance photosynthesis, *Plant physiology*, C Vol. 155 (1), pp: 93-100.
[132] Serafini, M. and Del Rio, D. (2004). Understanding the association between dietary antioxidants, redox status and disease: is the total antioxidant capacity the right tool?, *Redox Report*, C Vol. 9 (3), pp: 145-152.
[133] Cao, G. and Prior, R.L. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum, *Clinical chemistry*, C Vol. 44 (6), pp: 1309-1315.
[134] Teixeira, K.R., dos Santos, C.P., de Medeiros, L.A., Mendes, J.A., Cunha, T.M., De Angelis, K., Penha-Silva, N., de Oliveira, E.P., and Crispim, C.A. (2019). Night workers have lower levels of antioxidant defenses and higher levels of oxidative stress damage when compared to day workers, *Scientific reports*, C Vol. 9 (1), pp: 1-11.

APPENDICES

APPENDIX -5.1: ETHICAL COMMITTEE IN IRAQ



Kurdistan Regional Government
Ministry of Health
Slimani Directorate General of Health
Department of Planning
Scientific Research Division

Approval of Research Ethics Committee

Date: 29 September, 2019
Reference number: 2
Research title: **Determination of Serum total antioxidants and melatonin hormone levels on patients with breast cancer.**
Research name (s): **Amjad Mahmood Qadir**
Type of approval: Unconditional, taking into consideration the following mandates:

1) Obtaining the approval of Hiwa Hospital center in slimani Governorate.
2) The participants don't responsible for participate in funding any procedure(s) and /or investigation(s).
3) Health facilities are not responsible for the cost of any procedure(s) and /or investigation(s).
4) The participants have the right to withdraw from the study.
5) Confidentiality of the data should be secured.
6) Notifying the participant about the results of the procedure(s) and investigation(s) performed if applicable.
7) Any change in the methods of the study needs prior approval of Research Ethics committee.
8) Grantee of this letter is given the right to publish the results of the study.

[redacted] Dr.Sabah Nasraden Ahmad

[redacted] Dr.Safeen Rasul Ali

[redacted] Khalid Anwar Hama Ghareeb
Epidemiologist

KURDISTAN REGION M.O.H No:..... Date: / / D.O.H Sulaimaniya

[redacted] Jwana Jalal Rasheed
Epidemiologist

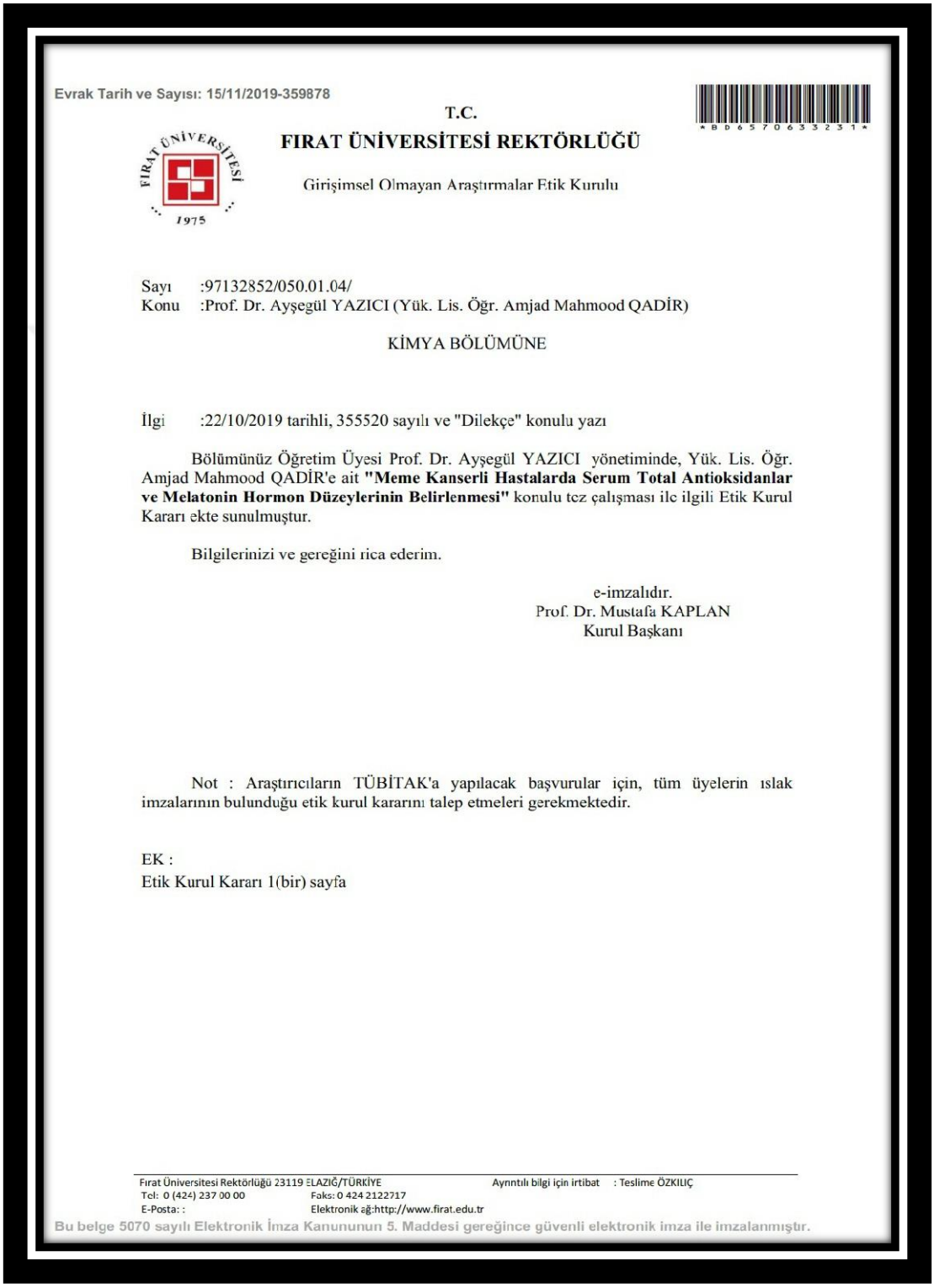
[redacted] Hawar Salar Aziz
Agriculture engineer

Address: Room 10, Floor 4
Slimani Directorate General of Health
Slimani, Kurdistan Region, Iraq

Contact:
Email: researchdoh.sul@g,ail.com

Figure A1. 1. Ethical Committee in Iraq

APPENDIX -5.2: ETHICAL COMMITTEE IN TURKEY



Evrak Tarih ve Sayısı: 15/11/2019-359878

T.C.

FIRAT ÜNİVERSİTESİ REKTÖRLÜĞÜ

Girişimsel Olmayan Araştırmalar Etik Kurulu

Sayı :97132852/050.01.04/
Konu :Prof. Dr. Ayşegül YAZICI (Yük. Lis. Öğr. Amjad Mahmood QADİR)

KİMYA BÖLÜMÜNE

İlgi :22/10/2019 tarihli, 355520 sayılı ve "Dilekçe" konulu yazı

Bölümünüz Öğretim Üyesi Prof. Dr. Ayşegül YAZICI yönetiminde, Yük. Lis. Öğr. Amjad Mahmood QADİR'e ait **"Meme Kanserli Hastalarda Serum Total Antioksidanlar ve Melatonin Hormon Düzeylerinin Belirlenmesi"** konulu tez çalışması ile ilgili Etik Kurul Kararı ekte sunulmuştur.

Bilgilerinizi ve gereğini rica ederim.

e-imzalıdır.
Prof. Dr. Mustafa KAPLAN
Kurul Başkanı

Not : Araştırıcıların TÜBİTAK'a yapılacak başvurular için, tüm üyelerin ıslak imzalarının bulunduğu etik kurul kararını talep etmeleri gerekmektedir.

EK :
Etik Kurul Kararı 1(bir) sayfa

Fırat Üniversitesi Rektörlüğü 23119 ELAZIĞ/TÜRKİYE Ayrıntılı bilgi için irtibat : Teslime ÖZKILIÇ
Tel: 0 (424) 237 00 00 Faks: 0 424 2122717
E-Posta: : Elektronik ağ:http://www.firat.edu.tr

Bu belge 5070 sayılı Elektronik İmza Kanununun 5. Maddesi gereğince güvenli elektronik imza ile imzalanmıştır.

Figure A1. 2. Ethical Committee in Turkey

APPENDIX-5.3: THE QUESTIONNAIRE FORM

Volunteer NO.	Date: / / 2019
Phone no:	Code:
Name:	Age: (Y)
Address:	Gender:
Economic situation:	Stage at diagnosis:
Marital status: married:	Single:
Time of sleep:	Time of awake:
Number of Children (gender and ages):	Age at first birth:
Length of time participant breastfed her children:	Date of diagnosis:
Finding:	
Breast cancer:	Normal:
Anthropometric measurement:	
Height: cm	Weight: Kg
BMI: Kg/m2	
Treatment history:	
Antioxidant supplements:	Hormone replacements:
Surgery:	Radiation therapy:
Chemotherapy:	
Disease history:	
Liver disease:	Chronic inflammatory disease:
Blood pressure:	Gastrointestinal diseases:
Kidney disease:	Thalassemia:
Diabetes:	Any diseases:
Risk factors:	
Family History:	Physical Activity:
Smoking:	Alcohol abuse

Figure A1. 3. The questionnaire form

APPENDIX- 5.4: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Figure A1. 4. High Performance Liquid Chromatography

APPENDIX- 5.5 ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Figure A1. 5. Enzyme Linked Immunosorbent Assay

CURRICULUM VITAE

[redacted]

PERSONAL INFORMATIONS

[redacted]

EDUCATION

Bachelor : University of Sulaimanyah, College of Science, Chemistry Department, 2011

High School : High school of Ahmad Mukhtar Jaf of Boys (83.5 0/0), Halabja, Iraq, 2007

WORK AND EXPERIENCE

- ✓ 2012-2013: Laboratory and practical experiences of Halabja Technical Institute:

  *Assistant Chemist in Biochemistry Laboratory
  *Assistant Chemist in Organic Chemistry Laboratory

- ✓ 2013-2014: Laboratory and practical experiences of Halabja Technical College:

  * Assistant Chemist in Biochemistry Laboratory
  * Assistant Chemist in Analytical Chemistry Laboratory

- ✓ 2015-2018: Laboratory and practical experiences of University of Halabja:

  *Assistant Chemist in Physical Thermodynamics Laboratory