CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY

BUCUREȘTI

FACULTY OF PHARMACY

DOCTORATE THESIS

SUMMARY

THE ROLE OF OXIDATIVE STRESS AND METABOLIC PRODUCTS IN CANCER PROGRESSION. THE ROLE OF IGF IN BREAST CANCER

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INTRODUCTION

Breast cancer (BrCa) in woman is the most frequent malignant tumor in incidence; about 1 in 8 women are diagnosed with BrCa during their lifetime. Most cases of breast cancer are combination of inherited genetic and factors that effect on these genes including metabolic syndrome (MetS), oxidative stress (OS), environmental factors and diet bring more light on BrCa initiation and progression and eventually opening a new era or adds more paths for management and cure.

The role of Insulin-like Growth Factor-1 (IGF1) and its receptor (IGF1R) in breast cancer progression was studied worldwide. IGF1/IGF1R pathway is involved directly or indirectly in breast cancer such as Proto-oncogenes (C-myc, int-2, c-erbB2 (HER2, neu, NGL) along with Cyclin D, tumor suppressor genes (BRCA1, BRCA2, p53) and DNA repair genes. Metabolic disorder including obesity, insulin resistance, D2M and CVD complications together constitute metabolic syndrome. In addition to changes in fuel availability and the adipocyte-derived hormones leptin and adiponectin. MetS and oxidative stress along with chronic inflammatory condition pave the way for pathologies such as carcinogenesis, aging, diabetes, obesity and CVD through altered cellular and nuclear mechanisms, including impaired DNA damage repair and cell cycle regulation.

Here in this study, we are investigating for the first time the network of IGF1/IGF1R, MetS metrics and oxidative stress products in BrCa progression through studding expression of IGF1/IGFR1 on BrCa tissues of women using immunohistochemistry staining and correlate the findings with the serum IGF1 levels of the same subjects. Then, measuring the levels of cholesterol, LDL, HDL, TG and Glucose with other metrics such as BMI, cigarette smoking and life style as indirect indicators for OS and to find out if there is a significant correlation between all findings and compare our results with similar data from reviewed literatures if applicable.

The objectives of this study focused on the clinical research through:

1. The relationship between IGF1/IGF1R, MetS and OS in BrCa progression by the link between IGF, blood glucose and lipid profiles in a population group of Syrian BrCa patients.

2. The association between Mets, OS and the IGF1/IGF1R expression, which stimulate cell BrCa growth and increase tumor size.

3. The serum IGF1 level as a prognostic factor predicting BrCa stage and lymph nodes metastases.

4. The correlation between serum IGF1 levels and the MetS metrics such as abnormal levels of Cholesterol, TG and Glucose.

5. The association between expression of IGF1/IGF1R and expressions of estrogen, progesterone and Her 2 receptors in breast tumors.

6. The Immunohistochemistry study of IGF1/IGF1R expression as novel biomarkers for diagnostic BrCa and cancer progression.

7. The blocking IGF1R by antibodies as a new strategy for treatment and management in Breast cancer

8. The relationships between leptinemia, insulin, and OS by utilizing the quotients of Insulin-to-Leptin Ratio (ILR) and Insulin-Adipogenic Resistance index (IAR-index) as potential biomarkers of the insulin-leptin axis, which illustrate the cross-talk between pancreatic β-cells and adipocytes.

9. The relation between antioxidant status and oxidative stress markers allow the initiation of preventive measures with regard to diet and therapy.

THEORETICAL ASPECTS

1. Insulin-Like Growth Factors –Key signal Molecule contribute in cancer progression

1.1. The IGF system

IGF system comprises a set of proteins that regulate vital cell processes in myriad tissues, including breast, colon, and prostate. The two growth factors, IGF-I and IGF-II, interact with six known IGF binding proteins (IGFBP), which regulate binding to the two IGF receptors (IGF-IR and IGF-IIR). The binding proteins are regulated by a group of IGFBP proteases and recently identified IGFBP-related proteins (IGFP-rP). (1,2,3)

1.2. Signal transduction through IGF-I , IGF-II and insulin receptors

IGF1, 2 and insulin are proficient to cross-bind to each other's receptor that leads to autophosphorylation of tyrosine kinase receptors and activates two signal transduction pathways. One is Ras/Raf/Mek/Erk/MAPK and PI3K pathways result in **cellular proliferation**. The second is via PI3K/Akt/mTOR pathway, which confers protection from **apoptosis** (4,5). AKT (PKB) is highly expressed in several human breast carcinoma cell lines (6,7), and promotes angiogenesis and **cellular survival** by activation eNOS (8). Akt mediated **metabolic effects** are induced by activation of enzymes involved in gluconeogenesis, glucose uptake, protein synthesis, and lipogenesis, the mTOR pathway mainly induces the cell growth responses. **(9)**

1.3. Interaction between IGFs and other growth factors in breast cancer

IGF-I/IGF-IR axis may interact with other growth factors in the BRCA milieu:

- TGFβ1 and IGF-I activate MMP that promos migration of breast tumors.
- IGF-I induced upregulation of VEGF-c and may play important roles in breast tumor progression and lymph node metastasis
- EGFR family: ERBB2 (HER2) may contribute to decreased IGF-IR expression during mammary tumorigenesis. IGF-I/HER2 crosstalk may occur via autocrine and paracrine signaling in Breast cancer.
- IGF-I, IGFBPs and ECM (vitronectin) have been reported to induce MCF7 survival, migration and invasion processes.(10)

1.4. Estrogen receptor (ER) and the IGF-I system in breast cancer

IGF-I/IGF-IR axis induces phosphorylation of ER (10,11), and induces rapidly and transiently methylation of ERα. Prostaglandin E2 along with HER2 and growth factors mediated

by IGF-I/IGF-IR axis through both AKT/MAPK pathways can enhance activity of aromatase (12). Primary invasive BRCA show that the expression IGFs are correlated with ER status and with menopausal status and BMI (13). ERα is reduced whereas ERβ is elevated resulting in increased phosphorylation of p38 MAPK and activation of the p53 substrate protein, causing apoptosis (14). A number of tumor suppressor genes (p53 and BRCA1) represses the IGF-IR promoter. Mutations in these tumor suppressor genes in Triple negative breast cancer (TNBC) are associated with elevated IGF-IR levels. IGF-IR is amplified in basal breast cancer, and high levels of IGF-IR protein are seen in basal breast cancers. (15)

2. METABOLIC SYNDROME AND BREAST CANCER

Metabolic syndrome (MetS) is a cluster of risk factors for CVD, diabetes and cancer. It constitutes a growing problem worldwide and have at least 3 of 5 of metabolic abnormalities including central obesity, dyslipidemia, T2DM, hypertension and hyperinsulinemia. (16)

2.1. Insulin and IGFs

Insulin induces transformation in normal breast epithelial cells and promotes proliferation of malignant breast cells. Higher levels of fasting insulin associate with breast cancer development (17). Insulin enhances the GH-stimulated synthesis of IGF-I by increasing the levels of GH receptors and by stimulating cellular uptake of amino acids for protein synthesis and increases the bioactivity of IGFI by inhibiting the synthesis of IGFBPs. (18,19)

2.2. The role of Hyperglycemia in Breast Cancer

Hyperglycemia has a positive association with both the risk of cancer and cancer-related mortality for years. High glucose levels enhance the proliferation and migration of breast cancer cells through stimulating EGFR activation and the Rho family such as GTPase Rac1 and Cdc42 mediate the corresponding signaling induced by high glucose levels (20,21). It has long been known that tumor cells take up more glucose and convert glucose to lactate even in the presence of oxygen (7), which constitutes an advantage for growth being considered a cancer metabolic hallmark. Cancer cells develop an adaptive program in the case of hypoxic conditions (which is found in solid tumors) by increasing HIF1α, increasing expression of both GLUT1 and key glycolytic enzymes (HK & LDH) (22,23). Glycosylation is characterized by the enzymatic addition of carbohydrate structures (glycans) to secretory and membrane-anchored proteins and lipids, these changes in glycosylation are considered a hallmark of cancer. Glucose could induce

cancer progression by increasing expression of the OS-responsive gene, thioredoxin-interacting protein, and subsequent increased levels of ROS. (24,25)

2.3. The relation between Lipid profile, IGFs and Breast Cancer

Elevated cholesterol was consistently associated with overall BrCa risk. IGF-I play mechanistic role through decreasing the expression of genes which contribute to the establishment of MetS involved in:

- lipid metabolism (ATP-citrate lyase, acetyl-CoA acyltransferase 1B, acetyl-CoA acetyltransferase 1) (26)
- Transport (Both HMG-CoA reductase and synthase, LDL-related protein 1, proprotein convertase subtilisin/Kesin type 9), resulting in dyslipidaemia. (27)
- Cholesterol synthesis

IGF-I actions indirect through lipid clearance from the bloodstream through inhibiting GH (lipolysis on adipocytes) and FFA uptake by muscles. (28)

2.4. The relation between Obesity, IGFs and Breast Cancer

Breast cancer is strongly influenced by obesity (29), and bioavailable IGF-I also increases in the obese state. Obesity, the most common cause of insulin resistance, is increasingly recognized as a low-grade inflammatory state in which overproduction of certain molecules, such as FFA, adiponectin, IL-6, leptin, plasminogen activator inhibitor-1, TNF-α, and monocyte chemoattractant protein, can play a role in malignant transformation and cancer progression. Importantly, chronic hyperglycemia, sex hormones and increased oxidative stress contribute also to increased cancer risk. An alternative pathway by which obesity may increase cancer risk is via adipokines. Adipose tissue synthesizes adipokines such as leptin and adiponectin (30,31)

3. OXIDATIVE STRESS AND CANCER

Oxidative stress (OS) is an imbalance of free radicals and antioxidants in the body, such as ROS, RNS, (O2-.), (OH•), (OH•) and ($^1O_2$)

3.1. Oxidative stress sources

There are an endogenous of OS sources (mitochondria, peroxisomes and microsomes), and exogenous sources such as diet, tobacco smoke, obesity, lifestyle, industrial chemicals, drugs (including anticancer drugs and, environmental factors (pollution, radiation, and UV rays). (32)

3.2. Antioxidants

Antioxidants are molecules that can donate an electron or remove free radicals without making themselves unstable (33). Antioxidants nonenzymatic sources (vitamins E, C, A, selenium, zinc,..etc) and enzymatic antioxidants include Cu/Zn, Mn superoxide dismutase, catalase, glutathione peroxidase and clutathione reductase (34)

3.3. Effects of oxidative stress at cellular level

ROS production with low levels are required to maintain physiological functions (proliferation, host defense and signal transduction). (35,36)

While ROS excessiveness leads to oxidative damage to DNA, proteins (Structural, enzymatic) and lipids; by reacting with the nucleic acids attacking the nitrogenous bases and the sugar phosphate backbone and can evoke single- and double-stranded DNA breaks (37,38). There are several markers of oxidative damage such as 8-hydroxy-2-deoxyguanosine (a marker of DNA oxidation), malondialdehyde and 4-hydroxynonenal (markers of lipid peroxidation) and protein carbonyl groups (a marker of protein oxidation) (36)

3.4. Oxidative stress involvement in Breast Carcinogenesis

The OS excessive may cause gene mutations or affect to intracellular signal transduction and transcription factors directly or via antioxidants, driving to carcinogenesis. Cancer cells, which exhibit an accelerated metabolism, demand high ROS concentrations to maintain their high proliferation rate (39). ROS overproduction has been detected in cancers cells due to high metabolic activity, peroxisomal activity, cellular signaling, oncogene activation, mitochondrial dysfunction and increased enzymatic activity of cyclooxygenases, oxidases, lipoxygenases, and thymidine phosphorylases. Cells maintain intracellular homeostasis through the immense antioxidant system, important antioxidant glutathione and transcription factor Nrf2 which contribute in balancing oxidative stress. ROS mediated signaling pathways stimulate pro-oncogenic signaling which eases angiogenesis, cancer progression, and survival. Concomitantly, to maintain ROS homeostasis and avoid cancer cell death, a high level of antioxidant capacity is associated with cancer cells (40). It is well known that cancer cells show constantly high levels of ROS due to oncogenic transformation including alteration in genetic, metabolic, and tumor microenvironment. (41)

ORIGINAL SECTION

4. MATERIALS AND METHODS

4.1. Study Population

The clinical study involved126 women with breast lesions classified into 3 groups

- 61 women with invasive ductal carcinoma (IDC) or ductal carcinoma (DCIS)
- 30 women with benign breast tumors (Fibroadenomas, Lipoma)
- 35 women with no tumors. This group was added as a control group.

4.2. Biological Sample Types

Tissue breast tumors specimens and Blood samples were used in this study; the tissues were formalin fixed paraffin embedded (FFPE).

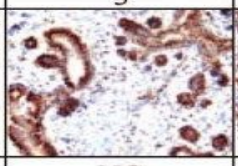
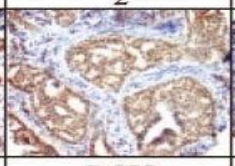
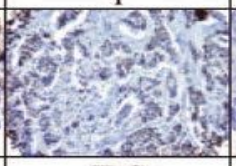
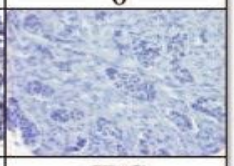
4.2. Biochemical methods

The IMMULITE® 1000 system was used to analyze the levels target analyets from the serums of the collected subjects such as IGF1, HDL, TG, LDL and Glucose. BMI was calculated as weight in kilograms/ [height in meters]^2, and standard clinical cutpoints categorized BMI as normal BMI < 30 kg/m2, and obese BMI ≥ 30 kg/m2.

4.4.Construction of TMA & Immunohistochemistry Analysis

Immunohistochemistry (IHC). Mouse monoclonal antibody against IGF1 and IGF1R were used to stain FFPE block.. IHC results were analyzed by scoring the intensity and percentage of areas of positive staining. Maximum positivity, which is the scoring at intensity of +++ was compared between each histological category. The tissue spots were scored using three (+++) for high intensity staining, two (++) for moderate staining, one (+) for low intensity and (–) for as a control group table (4.4.1).

Table 4.4.1. Scoring Method

Intensity	High	Moderate	Low	Negative
Strength	+++	++	+	–
Grade	3	2	1	0
Intensity of Color				
Histology	NG	DCIS	IDC	IDC

5. Study regarding the serum and tumor expression of IGF1/IGF-1R in subjects

5.1. Introduction and objectives

IGF-1 plays as mediator between the metabolic syndrome, oxidative stress and breast cancer progression. IGF1 and IGF2 through binding their receptors and other tyrosine kinase receptors induce signaling networks leading to fundamental cellular processes, such as cell growth, proliferation, differentiation and survival. Aberrations in the generation or action of IGFs play an important role in several pathological conditions (metabolic disorders and cancers). Redox pathways involving RNO and ROS involve in the pathogenesis. ROS and RNS demonstrate to alter IGF production and/or action. Therefore, we are aiming to study the correlation between IGF-I and some parameters according to MetS and excessive OS. In addition to expression and function of the IGF1/ IFG1R on BrCa progression of and their associations with MetS and other lipid profile parameters. The objectives of this investigation including:

1. The relationship between IGF1/IGF1R, MetS and OS in BrCa progression by the link between IGF, blood glucose and lipid profiles in subjects.

2. The association between Mets, OS and the IGF1/IGF1R expression, which stimulate cell BrCa growth and increase tumor size.

3. The serum IGF1 level as a prognostic factor predicting BrCa stage and lymph nodes metastases.

4. The correlation between serum IGF1 levels and the MetS metrics such as abnormal levels of Cholesterol, TG and Glucose.

5. The association between expression of IGF1/IGF1R and expressions of estrogen, progesterone and Her 2 receptors in breast tumors.

6. The Immunohistochemistry study of IGF1/IGF1R expression as novel biomarkers for diagnostic BrCa and cancer progression.

7. The blocking IGF1R by antibodies as a new strategy for treatment and management in Breast cancer.

5.2. Results

This study was conducted on 126 Syrian women with breast lesions:

- **Malignant group** of 61 women with IDC or DCIS.
- **Benign group** of 30 women with benign breast tumors
- **Healthy group** of 35 healthy women with no tumors

5.2.1. Demographic and clinical characterization of the population study group

Tumor type distribution: The malignant group was 60% of the specimens that means almost half of them, and benign group was 30% while 30% was healthy group.

Age group distribution: Women with the age group between (**41-50**) years represent 27.8% of the whole group followed by age group of (51-60) that represent 23%. For both age groups (31-40) and (61-70), each represents 5.9 % of the whole group, and for those whom are less than 30 years old (21-30) and (less than 20) they represent 14.3% and 3.2% of the whole group respectfully. The mean age for those who have malignant diseases was 51 and for those who have benign tumors was 39.

Educational level distribution: in our study we found that 87% **(P < 0.05)** of those subjects with malignant diseases have intermediate educational level or have never been to the school, (Figure 5.1).

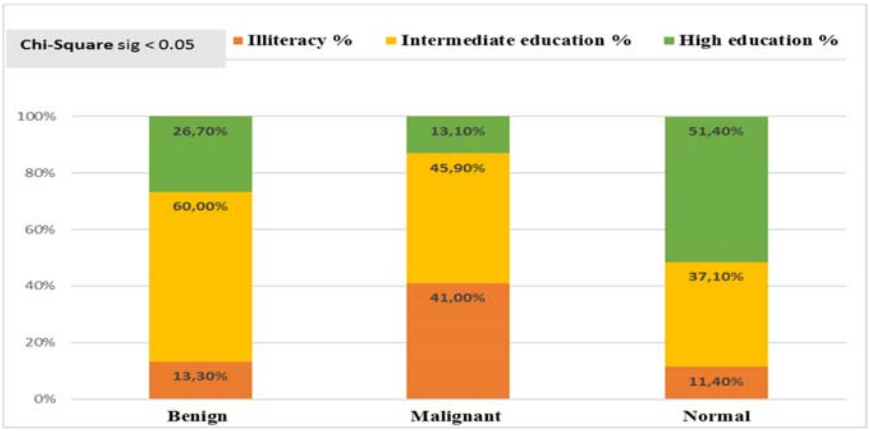


Figure 5.1. Distribution (%) of women according to their educational level

This is in fact very important observation that may contribute to the lack of preventive measures (self-examination, diet, environmental factors, etc..) that women may practice to decrease the risk of breast cancer progression, therefore they are losing early stage diagnosis.

Overweight group distribution: Our study revealed significant correlation between malignancies and body mass index (BMI). 77% of those with breast cancer are obese or overweight, (Figure 5.2)

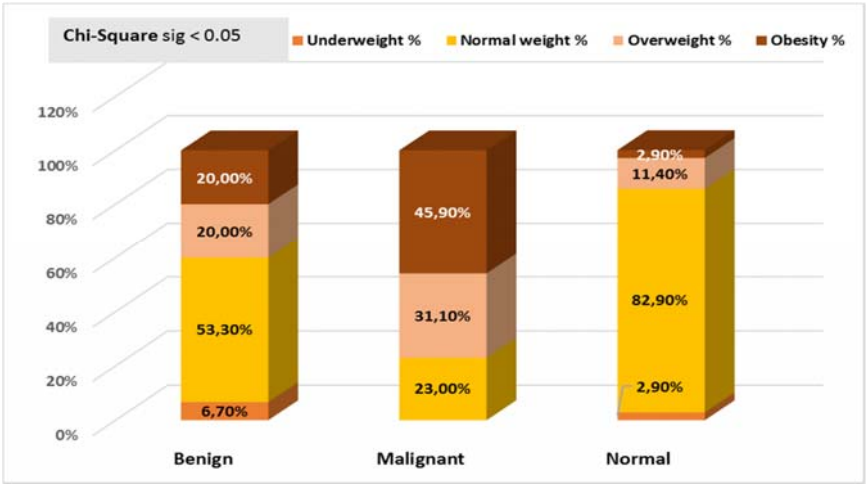


Figure 5.2. Distribution (%) of women according to their BMI

Cigarette smoking status: Our study found significant correlation between malignancies and cigarette smoking and 86 % of those with breast cancer are either smokers or secondary smokers, (Figure 5.3).

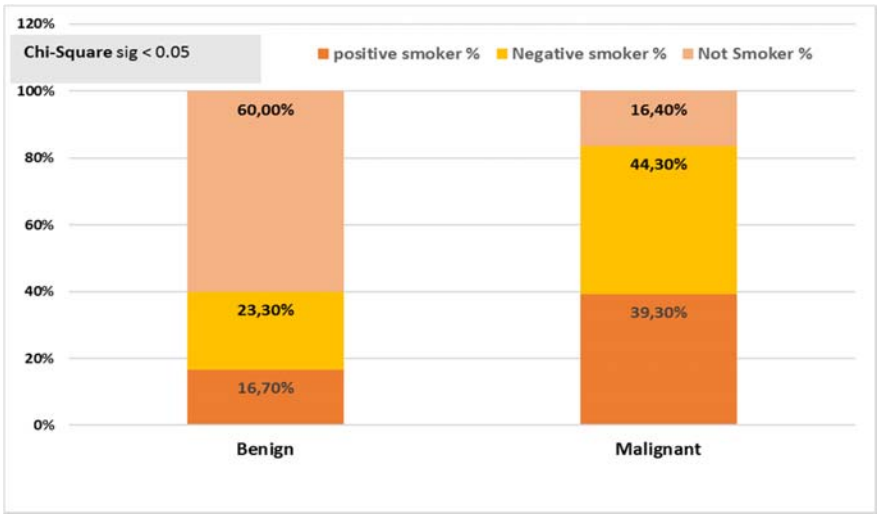


Figure 5.3. Distribution (%) of women according to the cigarette smoking

Metabolic characteristics: Our study results obtained from one-way variant analysis (ANOVA) that revealed significant differences between measurements of blood testing for TG, HDL, LDL, Cholesterol and Glucose for all subjects, (Figure5.4)

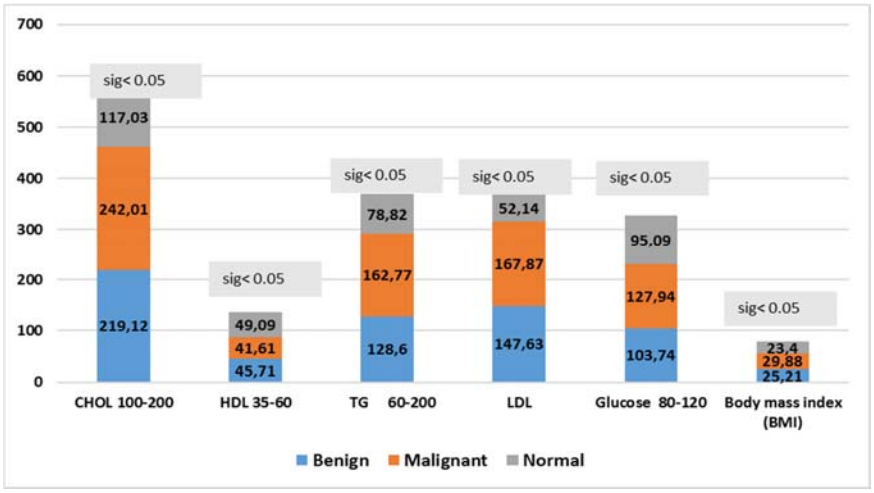


Figure 5.4. Serum levels of lipid profile, glucose and BMI values of all subjects

The **cholesterol** levels malignant group were higher than those with benign tumors and normal control ($P < 0.05$). Also, women with benign tumors showed higher cholesterol levels than those with normal control.

Expectedly, the differences between **HDL** levels between women with breast cancer and those in control were significant (P <0.05). Lower levels of HDL were observed in cancer patients. No significant differences were seen between patients with benign tumors and normal control (P >0.050).

On the other hand, the triglyceride **(TG)** levels and the low density lipoprotein **(LDL)** levels in patients with breast cancer were higher than those with benign tumor and normal control. [Malignant Tumors > Benign Tumors (P < 0.05) and Benign Tumors > normal (P < 0.05)].

Nevertheless, with regard to Glucose levels, there was a significant difference between patients with breast cancers and those with benign tumors or normal control. Where the glucose levels in cancer patients with high IGF1 is ranked at range of high normal.

5.2.2. Serum IGF-1 analysis

Since the mean levels of serum IGF-1 is different between age groups, a new method (ANCOVA) of analyzing IGF1 levels in plasma of patients samples was applied, the differences in IGF-1 levels were significant (P < 0.05) between the three groups. The mean IGF-1 levels in serum for patients with breast cancer was higher than being tumors group. But there were no significant differences between those with benign tumors and others with normal control (Figure5.5).

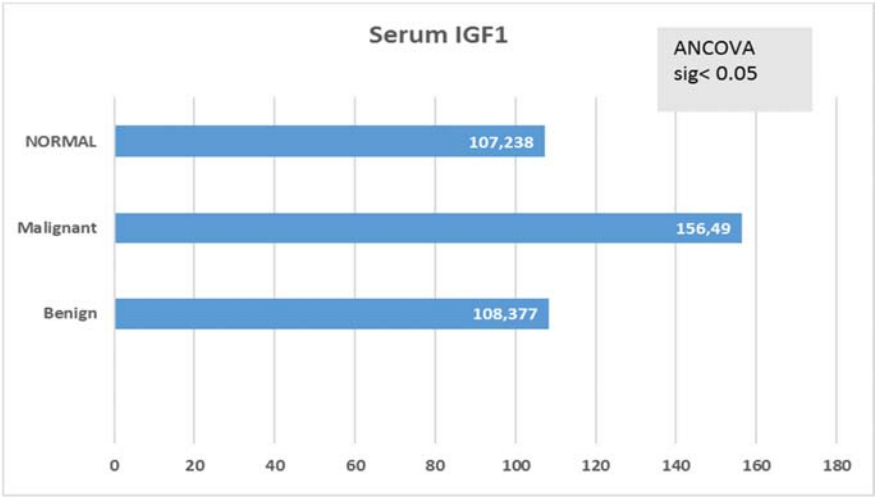


Figure 5.5. The mean serum levels of IGF1 in all study groups

5.2.3. Histopathology analysis of breast tumors

Immunohistochemistry staining for IGF-1 and IGF-1R was conducted on Tissue Micro Array. Statistical studies revealed that expression of IGF-1 was observed on 51.98 % on malignant samples vs 32.53 % on non-malignant samples

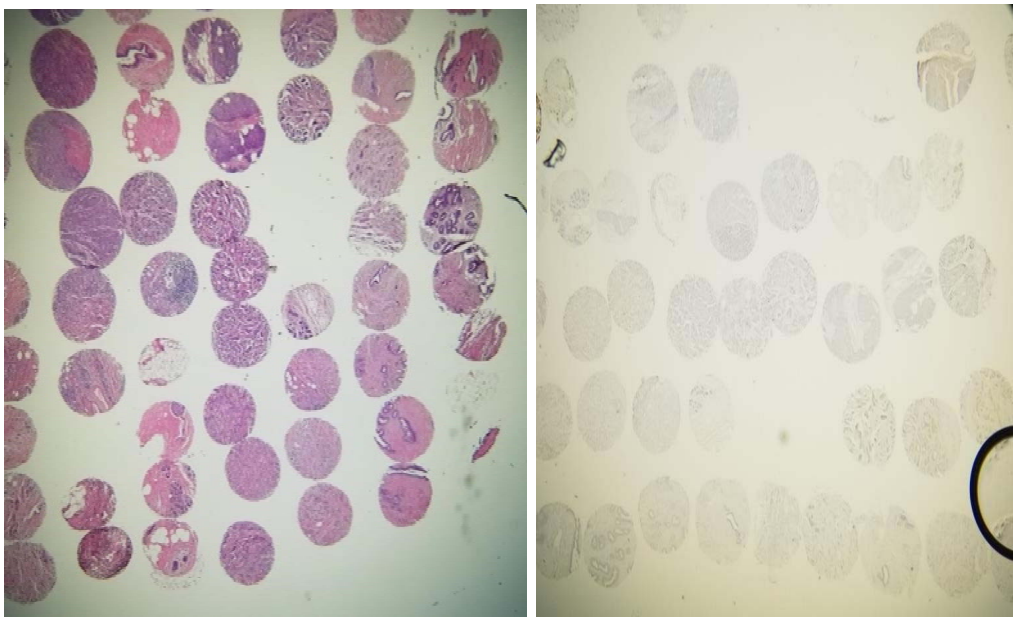


Figure 5.6. **A**. Portion of Breast Cancer Tissue Micro Array, H&E Stain X2

B. Portion of Breast Cancer Tissue Micro Array, IGF1, IHC staining (X2)

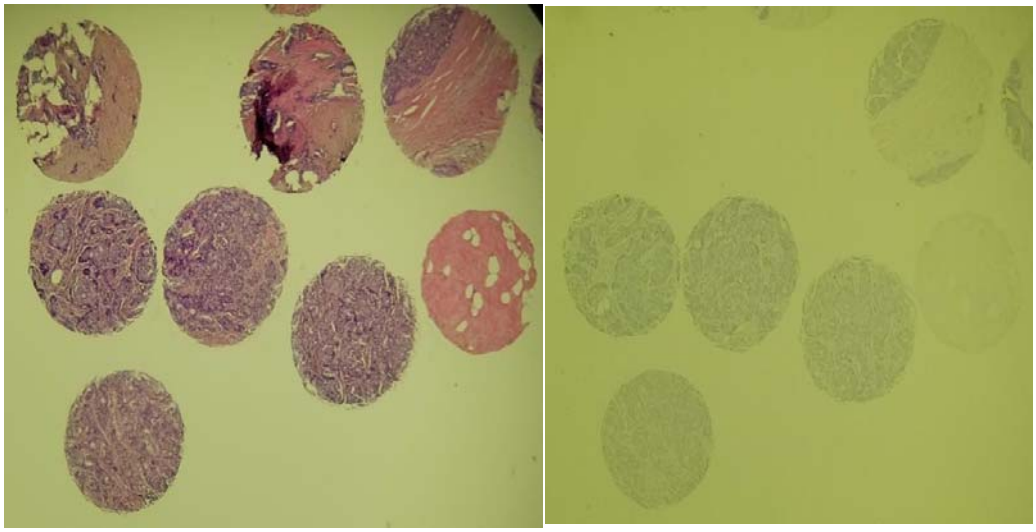


Figure 5.7 A.TMA, Invasiove Ductal Carcinoma (H&E X4)
B. IGF1, Immunostaining (+1) for the same sopts at A(X4)

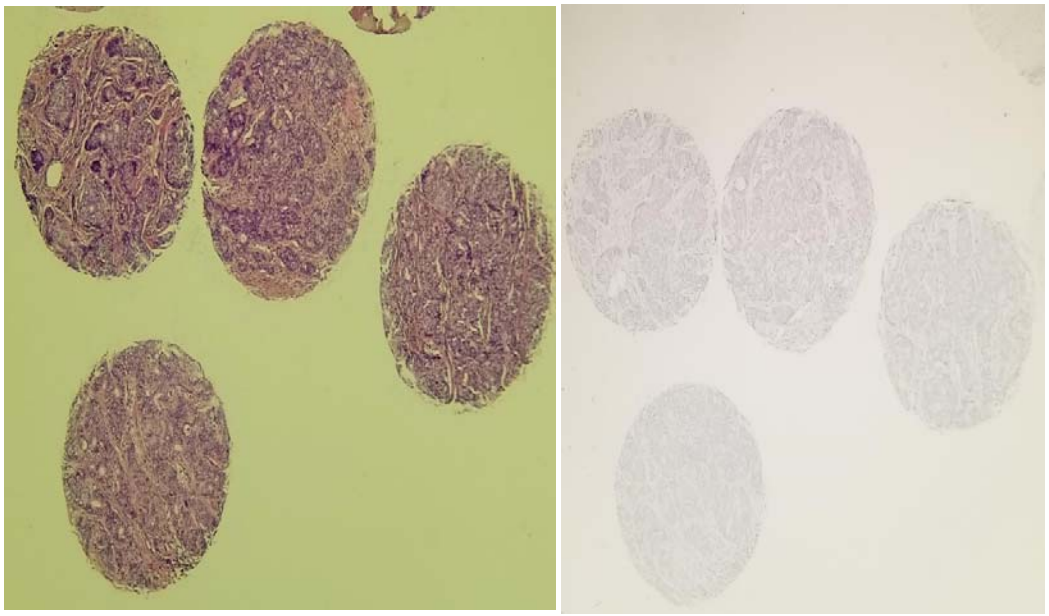


Figure 5.8. A. Same Spots as in figure 5.7. (A) (IDC) with power of (X10)
B. Same Spots as in (figure 5.7) (B) (IDC) with power of (X10) Showing IGF1, IHC staining (+1)

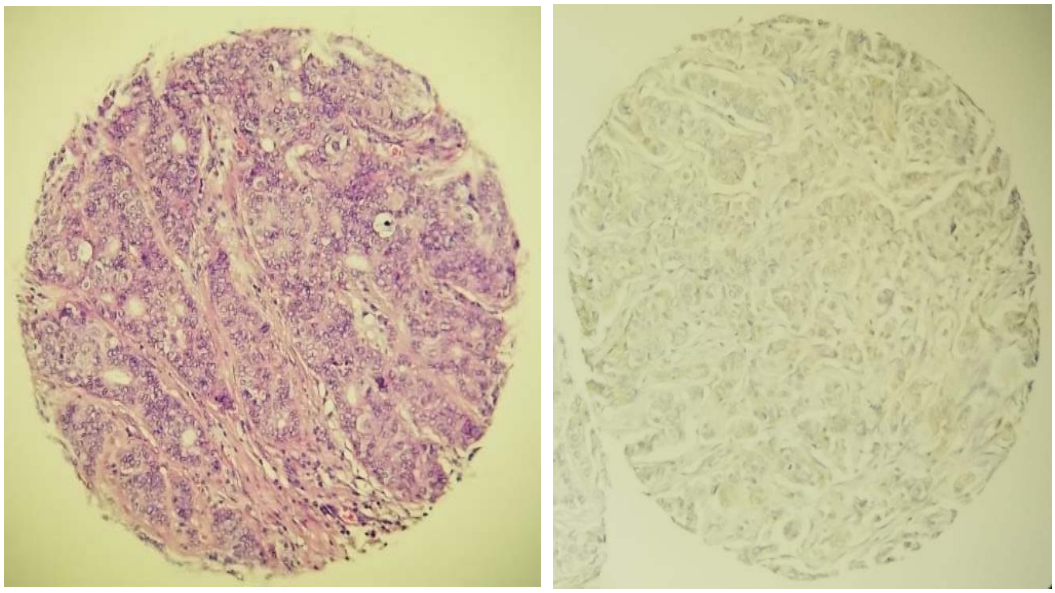
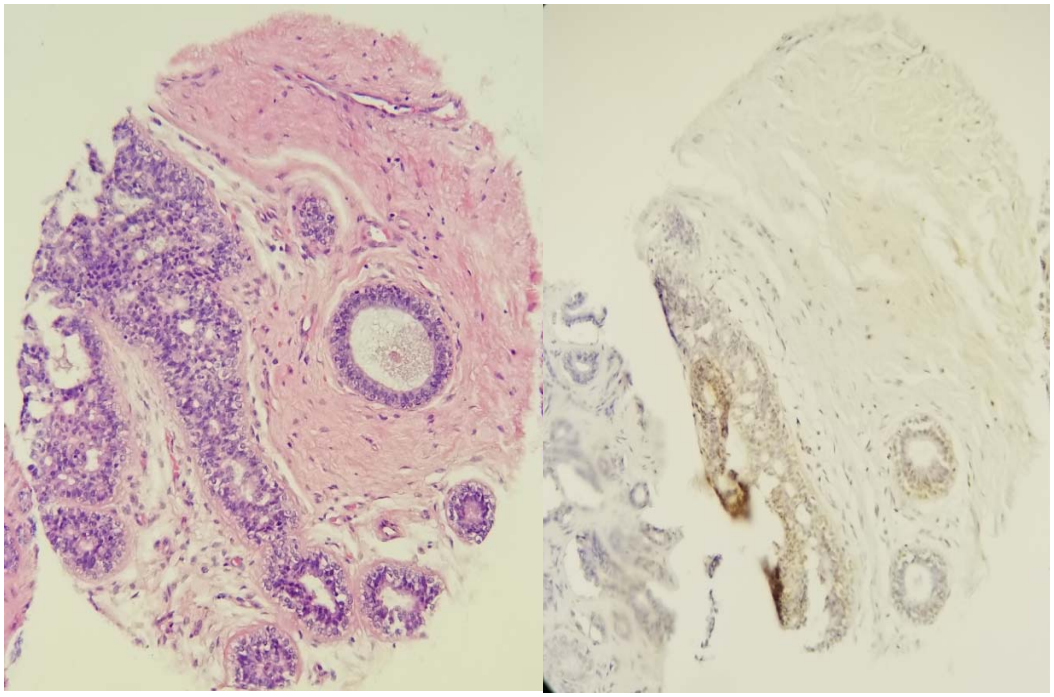
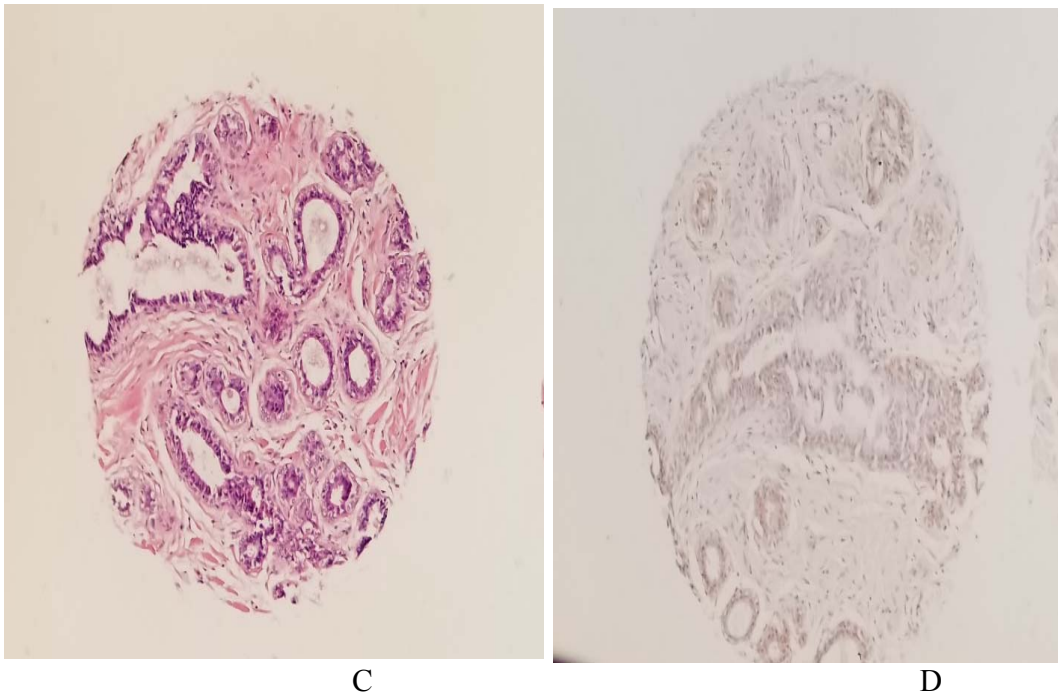


Figure 5.9. IDC with power of (X20). H & E stain and B. Showing IGF1, IHC staining (moderate 2+)



A B



C D

Figure 5.10.

A. DCIS with power of (X10). **B**. IGF1by IHC staining (moderate 2+)

C. Normal breast ducts (H&E) stain **D**. Mild immunostaining with IGF1(+1)

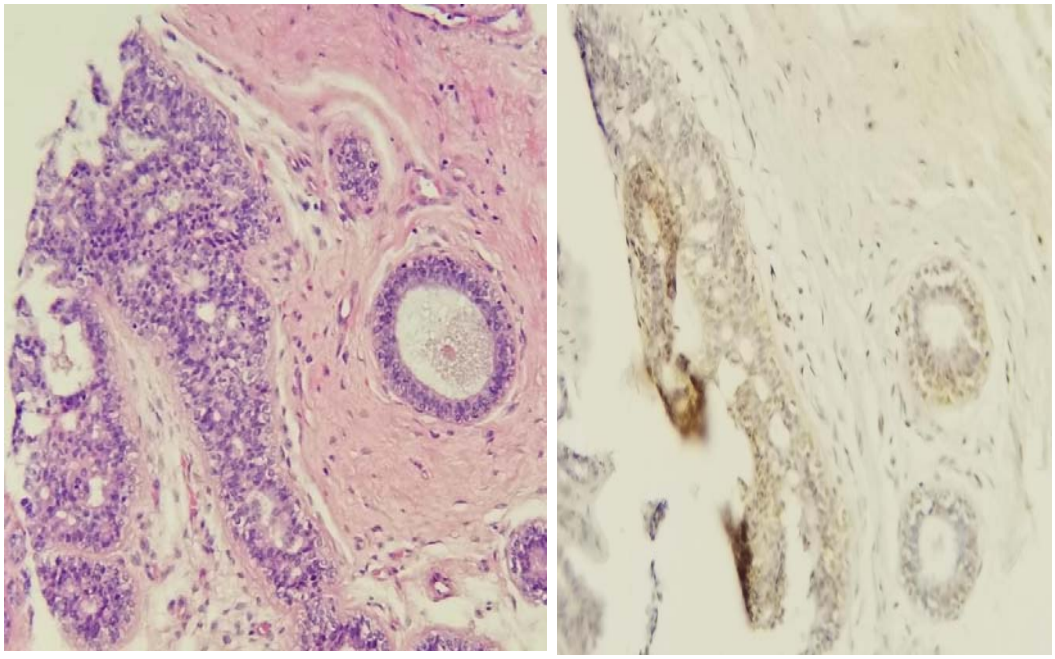


Figure 5.11. A. DCIS with with power of (X20) same as in figuer 5.10 A.
. B. Showing IGF1 IHC staining (modertae, 2+)

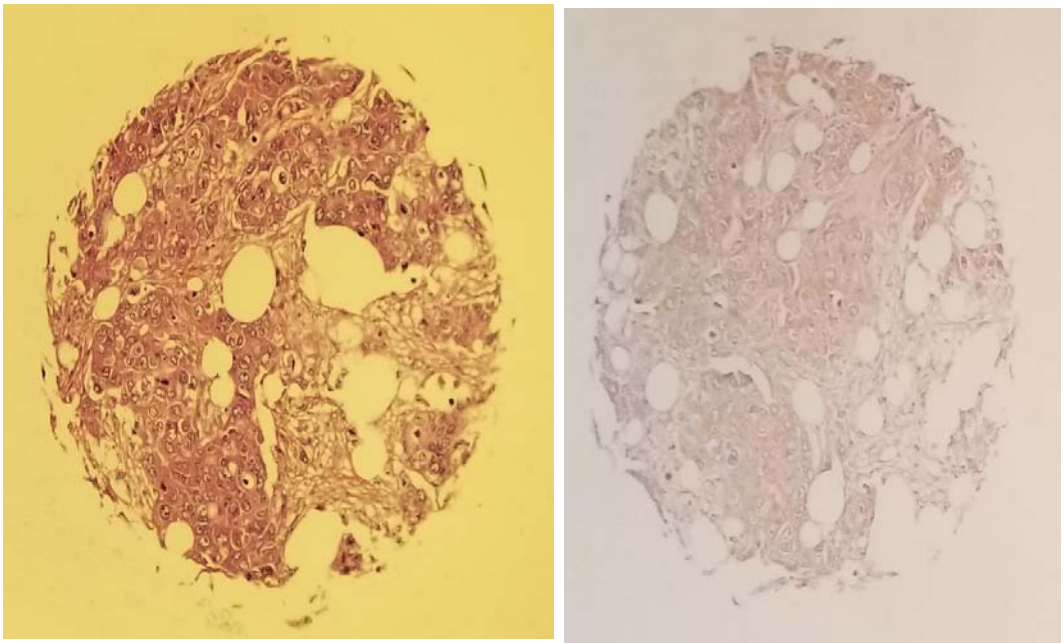
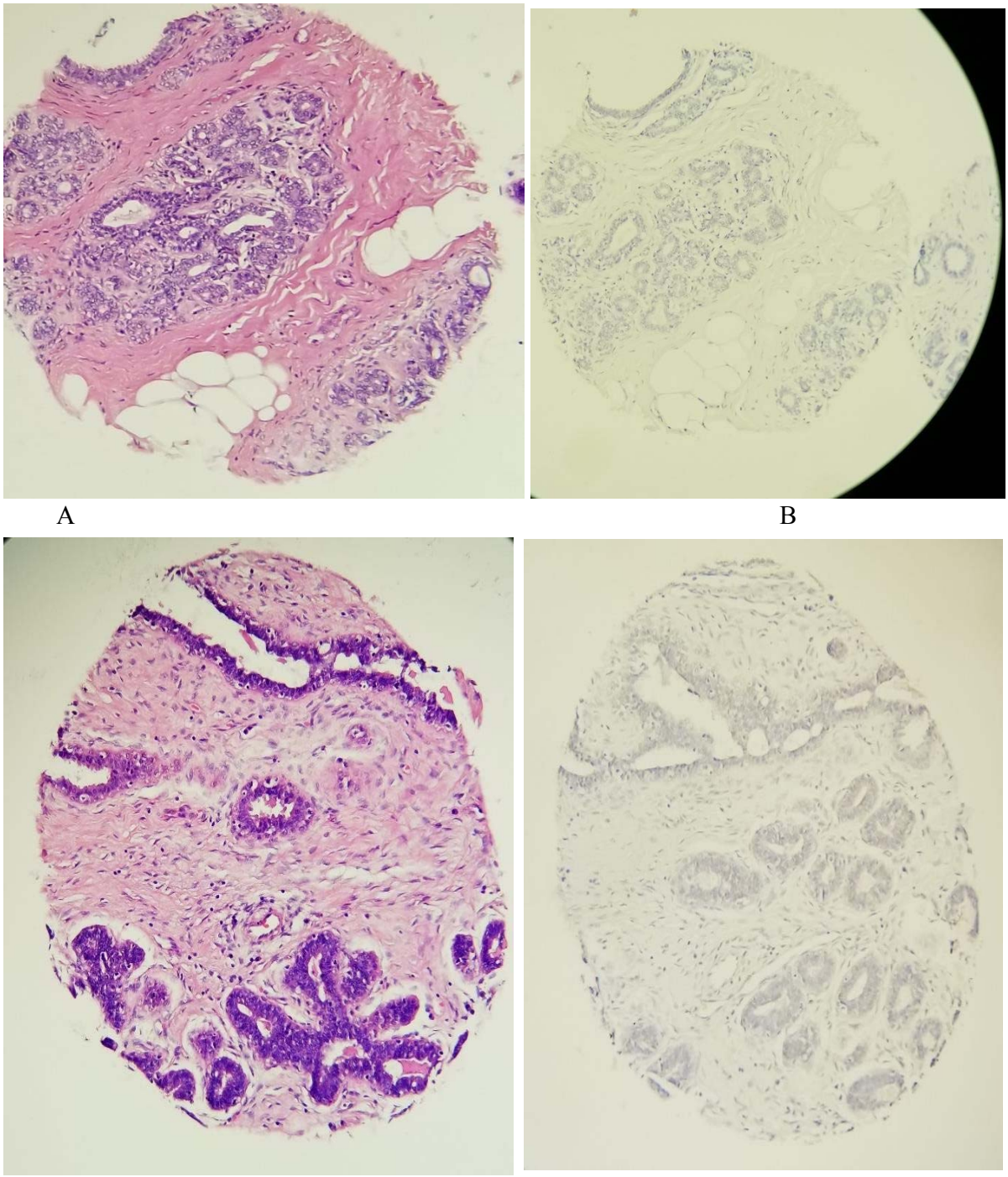


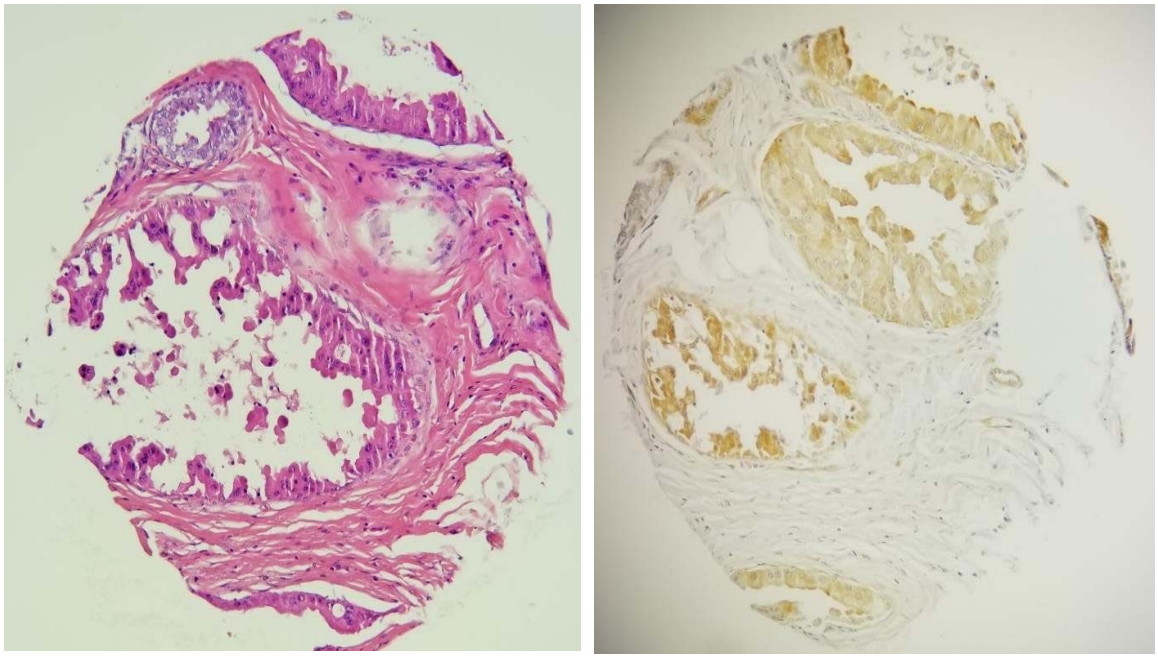
Figure 5.12. A. H&E Stain (X20) invasive ductal carcinoma of breast (IDC)
B. High Expression of IGF1 (X20) in IDC of breast same spot at A



A B

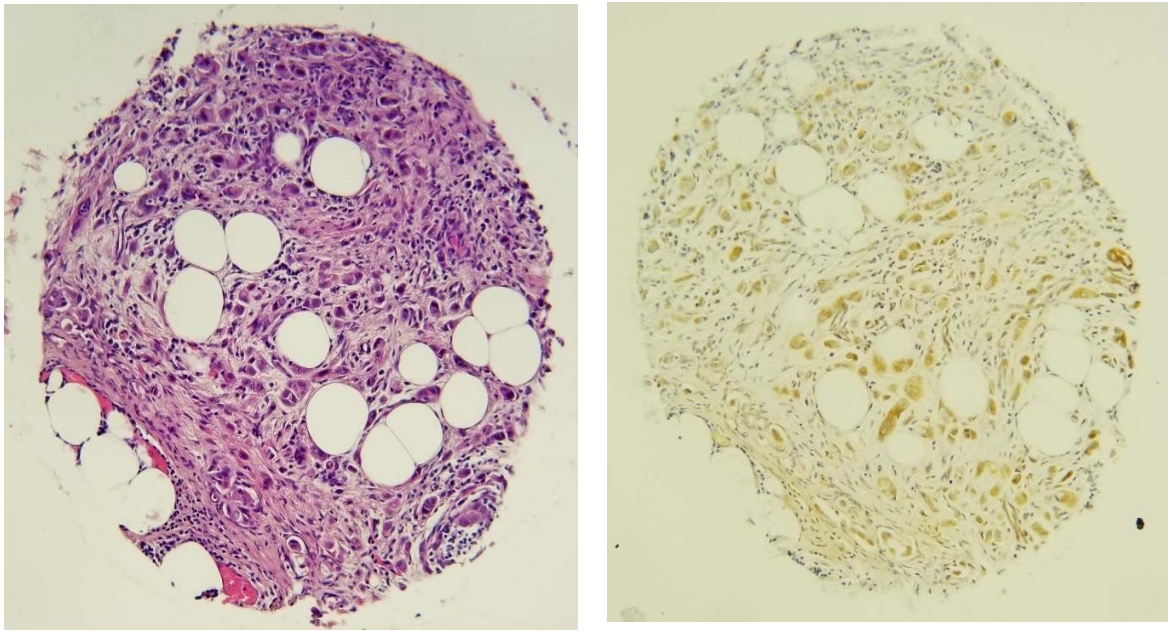
C D

Figure 5.13.
A.C. Normal Breast Tissue.
B.D. Negative immunostaining for IGF1. (X10)



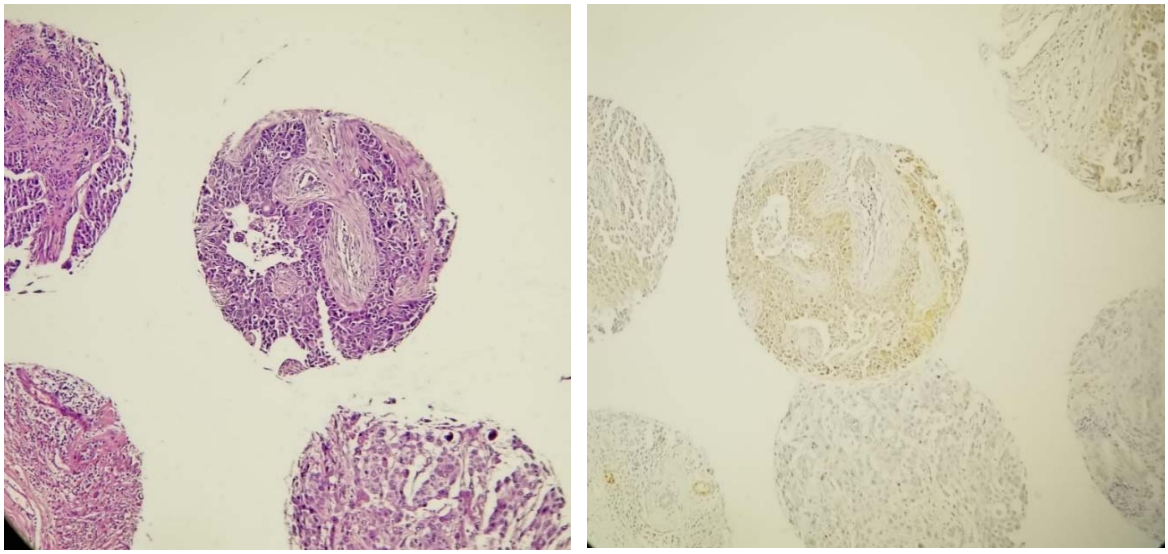
A B

Figure 5.14.
A. H & E stain (DCIS) with power of (X10) B. IHC staining for IGF1R (3+)



A B

Figure 5.15.
A. Invasive ductal carcinoma high grade with power of (X10)
B. Immunohistochemistry staining for IGF1R (3+) (X10)



A B

Figure 5.16.

A. Invasive ductal carcinoma, high grade with power of (X10).

B. IDC, high grade, IHC staining for IGF1R (3+) (X10)

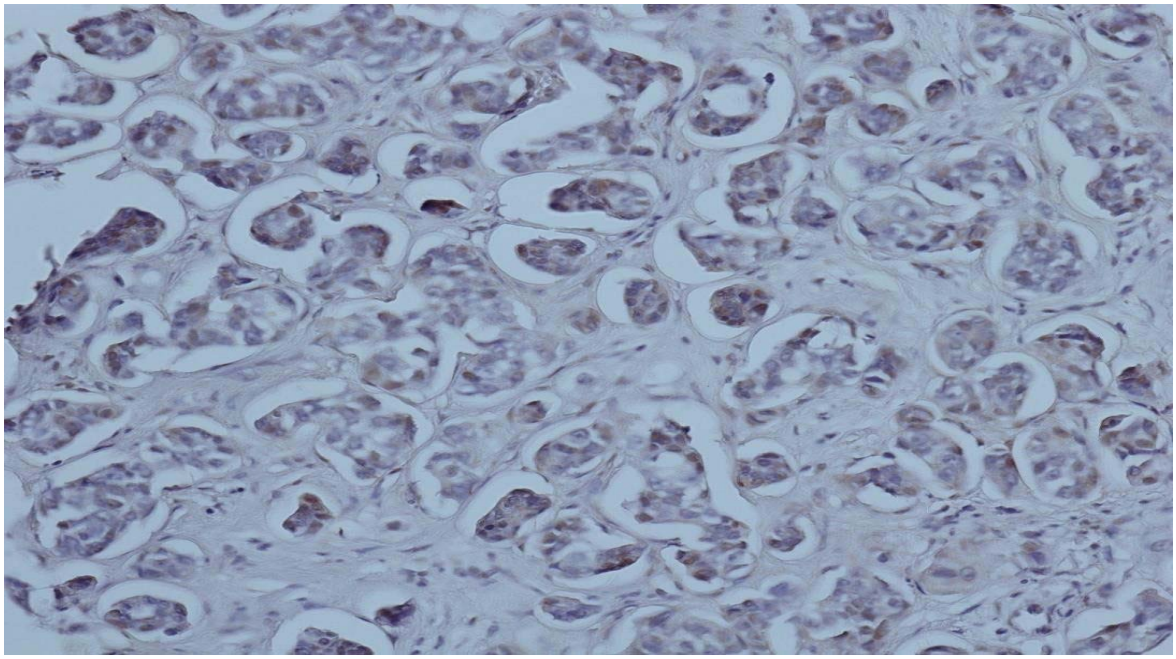


Figure 5.17

Invasive Ductal Carcinoma, high grade, IHC staining for IGF1R (3+) (X20)

Expression of IGF1 in breast cancer samples compare to the nonmalignant tumors was significant (P <0.05) according to TMA (Figure 5.18.)

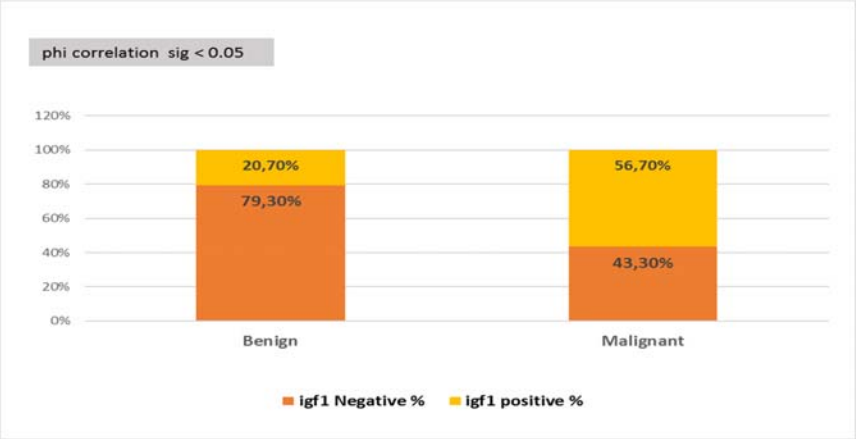


Figure 5.18. Expression of IGF-1 in Breast Tumors

statistical studies revealed that expression of IGF1R was observed on 56.35 % on malignant samples vs 23.8 % on non-malignant samples. IGF-1R Expression in breast cancer samples compare to the nonmalignant tumors was significant (P <0.05) (Figures.5.19)

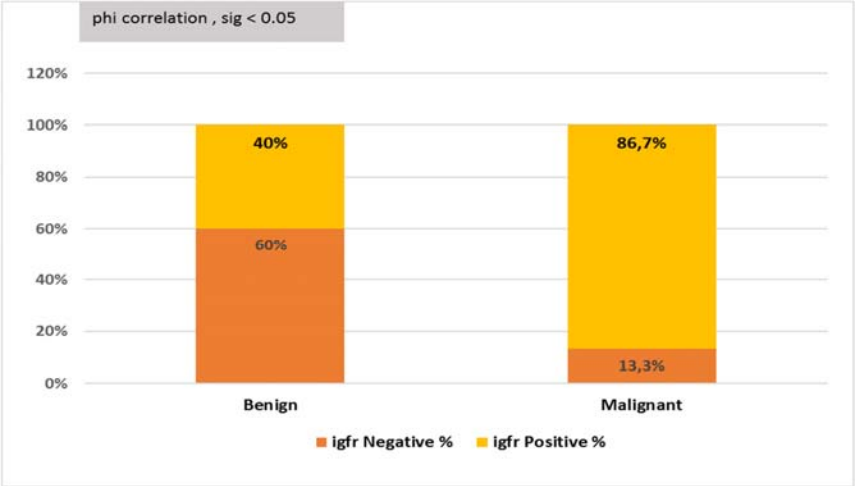


Figure 5.19. Expression of IGF-1R in Breast Tumors

Interesting findings that 70% of Her 2 positive breast cancer subjects expressed IGF1 while 54.1 % of patients with Her2 positive expressed IGF1 in their tissues (Figure5.20)

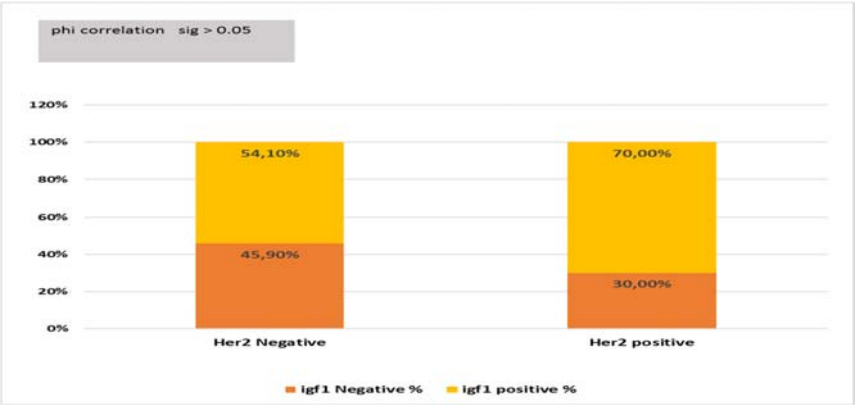


Figure 5.20. Expression of IGF1 and Her2 on TMA of Breast Tissues

Interesting findings that 80% of Her 2 positive breast cancer subjects expressed IGF1R while 20 % of patients with Her2 positive expressed IGF1R in their tissues (Figure 5.21).

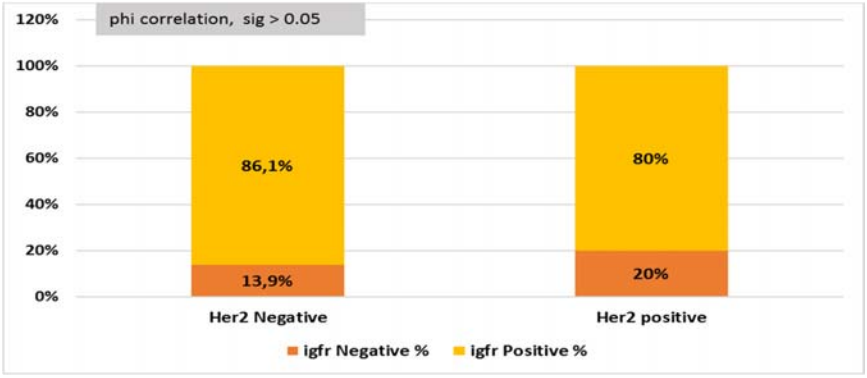


Figure 5.21 Expression of IGF-1R and Her2 on TMA of Breast Tissues

Importantly, about 61% of estrogen receptors positive (ER) breast cancer have IGF1 positive expression on their samples while 39% of patient with ER positive are IGF1 positive (Figure 5.22)

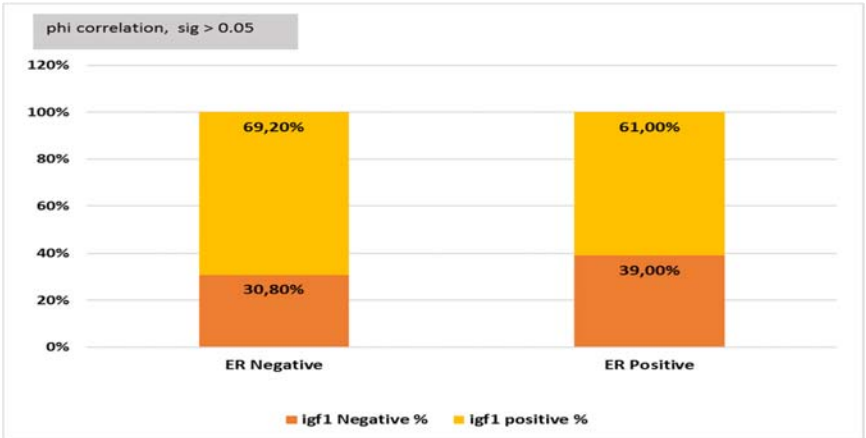


Figure 5.22. Expression of IGF1 and ER on TMA of Breast Tissues

Reviewing ER expression, it was found that 95% % of ER breast cancer samples are positive for IGF1R expression on their samples while 69.2 % of patient with ER positive are IGF1 positive (Figure 5.23)

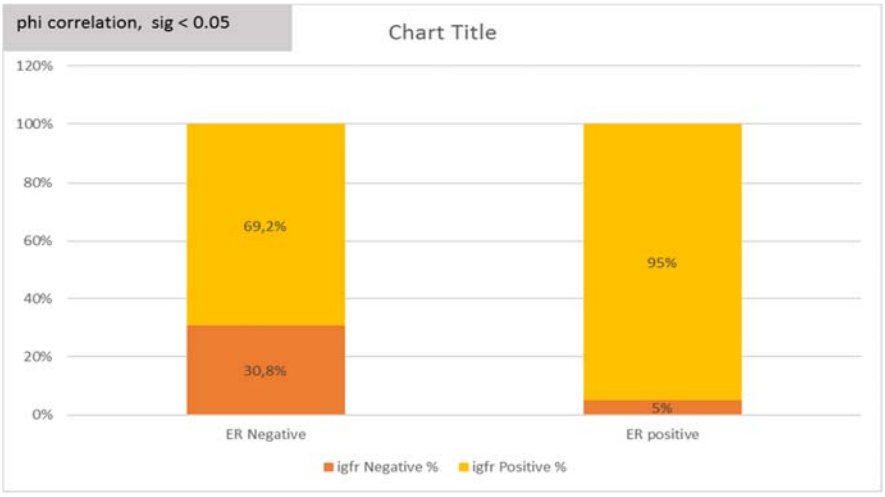
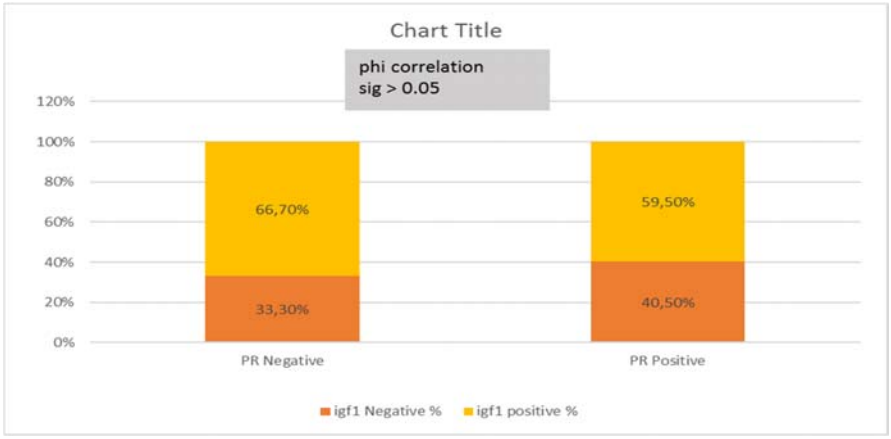


Figure 5.23. Expression of IGF1R and ER on TMA of Breast Tissue

On the other hand, 59.5 % of subjects that expressed IGF1 were positive for Progesterone receptors (PR) while 40.5% of patients with progesterone positive expressed IGF1 (Figure 5.24)

Figure 5.24. Expression of IGF1 and PR on TMA of Breast Tissue



For progesterone expression, 92.9 % of subjects that expressed IGF1R were positive for Progesterone receptors and 63.6 % of patients with progesterone positive expressed IGF1R (Figure 5.25)

Figure 5.25. Expression of IGF1R and PR on TMA of Breast Tissue

Our study revealed no correlations was found between triple negative breast cancer (TNBCs: ER, PR and Her2) and expression of IGF1. In addition, this study revealed no correlations was found between triple negative breast cancer and expression of IGF-IR.

It has been reported that IGF1 was involved in risk of early-onset of breast cancer in young women from hereditary breast cancer families and (IGF1) genotype predicts breast volume after pregnancy and hormonal contraception (42).

In our study, we did not find significant link between malignant tumor size and levels of IGF1 in serum (Figure 5.26). In addition, there was no significant association between the expression levels of IGF1 or IGF1R in tissue and tumor size (Figure5.27)

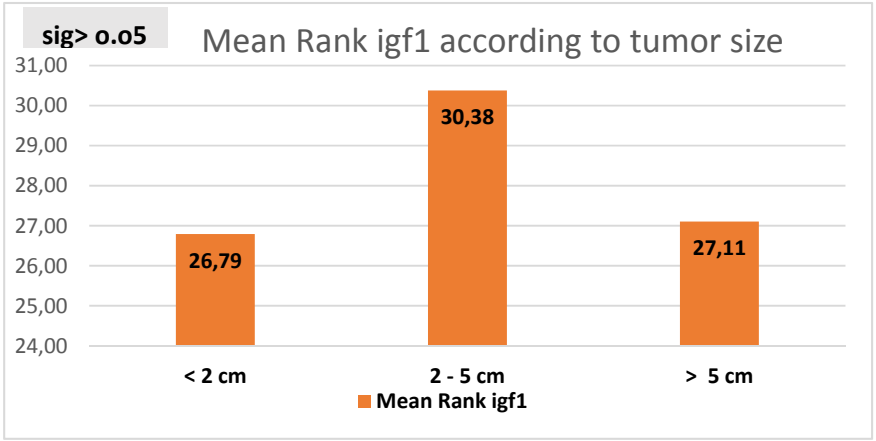


Figure 5.26. Tumor size according to Serum IGF1 levels

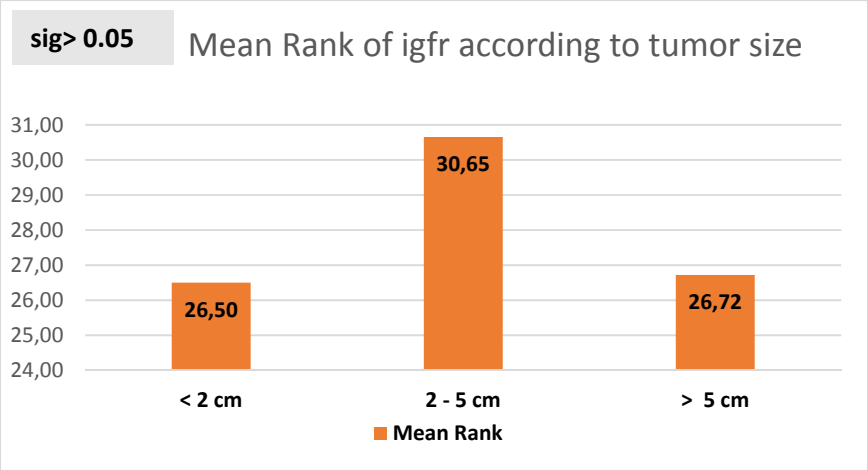


Figure 5.27. Tumor according to Serum IGF1R in Tissue

Studying the results between IGF1 expression and tumor stages according to lymph node metastases in our studies revealed **no significant** association was present between IGF1 expression levels and tumor stages.

About 68.2 of subjects with lymph nodes metastases have IGF1 levels above the mean and about 67.6 % with negative lymph nodes metastases have IGF1 levels above the mean (Figure 5.28)

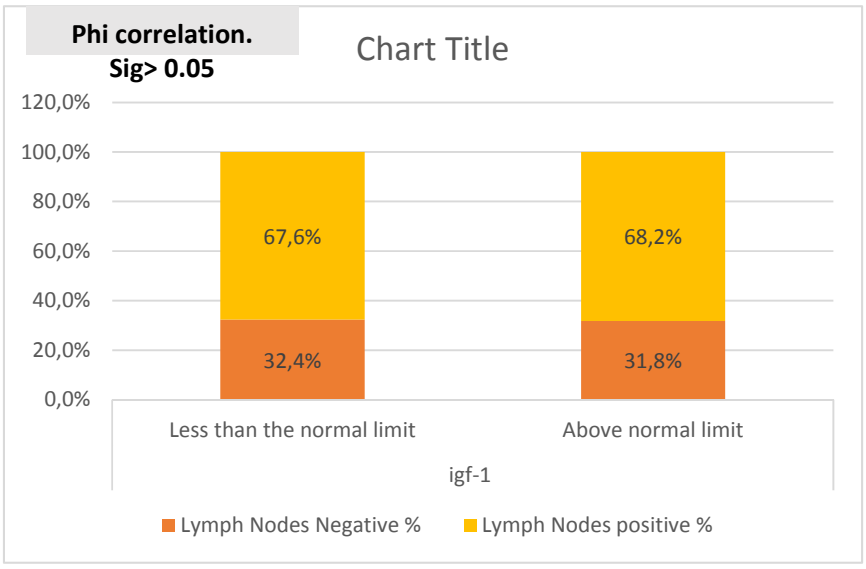


Figure 5.28. Lymph Nodes according to IGF1 Levels

5.3. Discussions

As mentioned in the introduction, Breast cancer is the most common cancer and the leading cause of cancer death among females worldwide, accounting for 25% of all cancer cases and 15% of all cancer-related deaths among females. (42)

Substantial evidence indicates that the socioeconomic status (SES) of breast cancer patients has a significant impact on prognosis through its associated influence on the cancer stage at diagnosis. Previous findings suggest people with lower incomes have a later cancer stage at point of diagnosis and a worse overall prognosis. Also, Socioeconomic status is significantly associated with education level and occupation, both of which can greatly influence patients' perception of the tumor, thereby affecting the level of early detection, diagnosis, and treatment. (42)

This observation was noted in our study and we found that 87% ($P < 0.05$) of those subjects with malignant diseases have intermediate educational level or have never been to the school. This is in fact very important observation that may contribute to the lack of preventive measures (self-examination, diet, environmental factors, etc..) that women may practice to decrease the risk of breast cancer progression, therefore they are losing early stage diagnosis.

Several studied linked between the obesity, IGF1 status and MetS as a phenomenon in BrCa progression. Obesity disorder is characterized by an increase in white adipose tissue mass. Clinically it is defined as a BMI of ≥30 kg/m2, however this definition fails to take into account gender, differences in muscle mass, and the relative amounts of peripheral and central fat in an individual subject.

Our study revealed significant correlation between malignancies and body mass index (BMI). 77% of those with breast cancer are obese or overweight.

Insulin resistance and other features of the MetS commonly accompany obesity. However, the degree of peripheral and hepatic insulin resistance varies between individuals, as does the presence of chronic inflammation, as evidenced by markers such as pro-inflammatory cytokines and CRP.

Expression of IGF1 in breast cancer was significantly associated with high BMI; it appears likely that sensitivity to insulin in obesity plays a central role in the GH-IGF axis response, by inhibiting pituitary GH, increasing hepatic GH responsiveness and suppressing hepatic IGFBP-1 secretion. Our result revealed that there is a correlation between IGF1 and BMI

in all age groups. This is confirming that obesity is associated with an increased IGF-I response to GH and increased GH-binding protein levels so that an increase in expression of GH receptor may explain lack of suppression of total IGF-I levels. Some studies report an inverse relationship between total IGF-I concentrations and measures of adiposity, such as waist circumference. (43)

In respect to the oxidative stress, that plays an important role in the pathogenesis of lung, breast and bladder cancer as well as chronic obstructive pulmonary disease, and atherosclerosis. Smoking may enhance OS not only through the production of reactive oxygen radicals in smoke but also through weakening of the antioxidant defense systems. In this study, we found significant correlation between malignancies and cigarette smoking and 86 % of those with breast cancer are either smokers or secondary smokers. (44)

However, this is may not be the case in other diseases such as rheumatoid arthritis, where is the cigarette smoking as an important cause of a relative IGF1 and leptin deficiency in RA patients. (45)

On the other hands, another study showed no relationship noted between tobacco smoking and either IGF-1 or IGFBP-3 overexpression. (46)

Variations in results between studies may contributed to different in samples size, geographic locations, individual genomic programs and life styles.

Elevations in LDL cholesterol and three glycerides levels with low levels of HDL cholesterol can predict development and prognosis in metabolic syndrome and eventually in cardiovascular diseases and malignant diseases in general. In our studies, the cholesterol and LDL levels in patients with breast cancers along with TG were higher in breast cancer patients than benign tumors and normal controls ($P < 0.05$). While lower levels of HDL were observed in cancer patients as expected ($P < 0.05$).

Nevertheless. Glucose levels and BMI, there was no significant differences between patients with breast cancers and those with benign tumors or normal control. Interestingly the patients with normal controls have glucose levels and BMIs higher than breast cancer group.

In summary, the mean levels of IGF1 in plasma/serum for patients with breast cancer was 156.49 ng/ml which is higher than those with being tumors and no significant differences between those with benign tumors and others with normal control.

From the above findings the indicators of metabolic syndromes (TG, Cholesterol, LDL, HDL) and remarks of oxidative stress (tobacco smoking and others) are associated with elevation or high expression of IGF1 which is eventually linked to breast cancer progression.

From the immunohistochemistry staining, we found that IGF1 and IGF1R showed high expression on malignant tissue compared to nonmalignant. The expression of IGF1 was observed on 51.98 % on malignant samples vs 32.53 % on non-malignant samples ($P<0.05$) and the expression of IGF1R was observed on 56.35 % on malignant samples vs 23.8 % on non-malignant samples ($P <0.05$)

It was found that IGF1R expression patterns in epithelial cells of normal tubular ductal lobular unit in benign breast biopsies were associated with an increased risk of subsequent breast cancer. (47)

In fact, receptor tyrosine kinases are transmembrane molecular scaffolds that influence cellular processes including the cell cycle, cell migration, cell metabolism survival, proliferation and differentiation. Insulin-like growth factor-I receptor (IGF-IR) is an RTK that stimulates growth in many different cell types, blocks apoptosis, acts as an intermediate of many growth hormone responses, and may stimulate the growth of some types of cancer. The IGF-IR associated with ligand insulin-like growth factor I (IGF-1) promotes association of IGF-IR with Shc, Grb2, and Sos-1, which initiates Ras and Erk kinase cascades, thereby modifying transcription factor activity, such as activation of the ELK transcription factors. Therefore, activation of IGF1R by IGF1 Ligand favor cellular proliferation and cancer progression. (48)

Interesting findings that 70 % of Her 2 positive breast cancer expressed IGF1 in their tissues and 80 % of Her 2 positive breast cancer subjects expressed IGF1R. Our results are similar to the previous published data for the same subject. (49)

Both, IGF1R and Her2 Receptors promote cellular growth after activations and they function on coordination with ER receptors as well.

Since, our result revealed that there is a correlation between IGF1 and ER Expression. It appears that the binding of IGF-I to its receptor activates the tyrosine kinase and initiates a cascade of auto-phosphorylation that activate intracellular kinases and nuclear transcription factors, including the ER. There is increasing evidence for a complex mechanism of crosstalk between peptide and steroid pathways. The emerging model of crosstalk between IGF-I and estrogens suggests that estrogens, acting through the ER, induce the expression of IGF1. IGF1

in turn exerts its actions through binding to the IGF1R, a transmembrane protein with tyrosine kinase activity.

On the other hand, the inhibition of IGF-1R signaling with anti-IGFR antibodies, May restricts BrCa cell growth in vivo.

Antiestrogens, on the other hand, inhibit IGF1 receptor dependent growth by downregulating the IGF autocrine pathway and modulating the expression of IGF binding protein. In addition, antiestrogens decrease the expression of IGF-I binding sites and suppress the activation.

In addition, inhibitors that blocked PKA and PI3K effect of IGF-I on ER-α expression and activity, suggesting that protein kinase and PI3K may be involved in the crosstalk between the IGF-I and ER-α pathways. Because the serine/threonine protein kinase Akt (PKB) is downstream PI3K, experiments were conducted to determine whether Akt is involved in the crosstalk between IGF-IR and ER-α. (50)

Since the progesterone is an ovarian steroid hormone that is essential for normal breast development during puberty and in preparation for lactation and breastfeeding. The actions of progesterone are primarily mediated by its high-affinity receptors, which include the classical progesterone receptor PR (a-b). In fact, PR action in BrCa is grossly understudied and remains controversial. (51)

Confounding the role of progesterone in BrCa is that progesterone has biphasic effects ((both proliferative and inhibitory) on the breast cancer cell lines grown in vitro. For this reason, it has been suggested that progesterone acts primarily as a priming agent, with growth promoting activity dependent upon cellular context. For example, progestins upregulate many of the components of growth factor-initiated signaling pathways, including IRS-2 and EGFR family members and their ligands, therefore, progesterone may act primarily by sensitizing BrCa cells to growth factor and cytokine signals. In addition, IGF1 my influence directly or indirectly the GH effects on many luteal functions. (52,53)

Nevertheless, the association of expression of IGF, IGF1R and progesterone have been reported in several studies and in our study, this association is overexpressed in tissue of BrCa patients that is in concordance with published data.

Recent studies have shown that the levels of estrogens and other steroid hormones in breast fluids are much higher than in serum, which may be the result of local synthesis or

increased uptake from the circulation. No differences in estrogen levels of breast fluid have been found between normal women and those with breast disease. A possible explanation may be differences in the levels of estrogen antagonists, such as progesterone.

Finally, we did not find any correlation between IGF1 serum levels and tumor stage or lymph nodes metastases as have been demonstrated in BrCa and other cancer types such as Subjects samples, age groups and geographic distribution as well as genotypes may explain this contradictory.

5.4. Conclusion

Our studies showed that the elevation of serum IGF1 levels as well as the overexpression of IGF1/IGF1R in tissues of BrCa patients are associated with high levels of MetS markers such as LDL, Cholesterol, TG, glucose, BMI and low levels of HDL. On the other hand, overexpressed of IGF1/IGF1R is associated with expression of Her2, Estrogen and Progesterone in breast cancer tissues and eventually adds more risk in cancer progression, that support the link of OS on breast cancer progression.

Interestingly, this study revealed remarkable link between malignant subjects and their educational levels, which may have reflected in many aspects on their lifestyles such as low physical exercises, diet type and high carbohydrates uptake that increase the risk of breast cancer.

Nevertheless, we did not find in this study any correlation between serum IGF1 levels and tumor stage or lymph nodes metastases in patient's breast tumors as have been mentioned in previously published reports in BrCa and other cancer types

6. Insulin-leptin axis, Cardiometabolic risk and Oxidative Stress in elderly with MetS

6.1. Introduction

This Study was conducted at the Department of Biochemistry at Davila University in support of our findings about the relation between MS, OS, aging and risk of CVD and cancer.

The study aimed to establish indicators for MS by revealing the Insulin-to-Leptin Ratio (ILR) and Insulin Adipogenic Resistance (IAR)-index.

As it has been reported, aging leads to a number of physiological changes in all organ systems, including changes in circulating hormone levels and inflammatory responses, increases in fat deposition, decreases in metabolism, reductions in growth factors and tendency to develop certain cancer types (54). Hence, individuals with MS have abnormal adipokine profiles that affect insulin sensitivity (55,56) and the MS triad of obesity, insulin resistance and hypertension prevalently increases with age, as has been reported in numerous worldwide studies (57,58), white adipose tissue is under influence of autocrine, paracrine, and endocrine signals (59). Through their complex signaling cascades, leptin and insulin are key playing metabolic hormones involved in the first line of the adaptive response mechanisms related to the food intake, glucose homeostasis and energy expenditure (60). Nevertheless, insulin also acts to regulate appetite by influencing the expression of different orexigenic and anorexigenic neuropeptides in the brain, ultimately decreasing food intake and body weight (61). Also, the leptin hormone secreted by adipocytes functions similarly to that observed for insulin, which effects both glucose metabolism and free fatty acid (FFA) oxidation in the liver, pancreas, and skeletal muscle (62).

At the molecular level, adipocytokines such as (IL-1, IL-6, IL-8, leptin, C-reactive protein (CRP) and plasminogen activator inhibitor 1 (PAI-1) accompanied by a decreased production of adiponectin (63,64).

In pancreatic islets, elevated glucose concentrations increase the metabolic activity of islet cells, exhausting them, and leading to elevated formation of ROS. (65)

6.2. Materials and Methods

MetS group (45 patients 37 women and 8 men) according to the criteria of AHA/NHLBI definition, while the control group (27 healthy women and 8 healthy men). The levels of fasting glucose, cholesterol, LDL-C, HDL-C, TG and serum uric acid were measured by standard laboratory techniques on Thermo Fisher Diagnostic System (USA). The levels of Fasting insulin and leptin were assessed with a Sandwich ELISA. The measurement of the Ferric Reducing Ability of Plasma (FRAP) was conducted using the method described by Jansen and Ruskovska (66). The serum Advanced Oxidation Protein Products (AOPP) were measured by spectrophotometer.

6.3. Results

The study revealed no significant difference in the age distribution between the study groups associated with metabolic syndrome, but as expected, BMI, systolic and diastolic blood pressures, TG, cholesterol and LDL cholesterol, as well as fasting glucose levels were significantly higher in subjects with MetS, compared with the control group. HDL-cholesterol levels were significantly lower in MetS group than in control. Hence, the calculated Insulin-to-Leptin Ratio (ILR) and the Insulin-Adipogenic Resistance index (IAR-index) were further used as proposed surrogate biomarkers to explore relationships between metabolic profile, oxidative stress and the secretory functions of β-pancreatic cells and adipocytes, in both groups.

With respect to serum antioxidant capacity, in both study groups we found significant positive correlations between FRAP and uric acid levels.

Overall, the CVD risk biochemical markers, oxidative stress and inflammation markers were higher in MS group, indicating a relevant atherogenic risk associated with hyperinsulinemia and hyperleptinemia. Furthermore, a significant positive association between fasting serum insulin and serum leptin levels was pointed out only in MetS patients. In apparently healthy subjects, leptin levels were significantly inversely associated with fasting glucose and LDL-cholesterol as well as with FRAP and uric acid

In MetS group, leptinemia was significantly negatively correlated only with fasting glucose. The other relationships identified in control subjects were no longer identified, neither for leptin nor for ILR individual values in MS patients.

Among the markers evaluated to assess the oxidative stress exerted at systemic level, in MS subjects the most relevant was AOPP which was positively correlated with triglycerides and negatively correlated with HDL-C as well with the ratio FRAP/UA.

By using the multivariate linear regression analysis, method stepwise, in which leptin, ILR and IAR-index were introduced as dependent parameters we pointed out that in control subjects, the determinant of leptin was fasting glucose. Whereas in MS subjects, insulin was the significant determinant of leptin. In control subjects, LDL and FRAP were the significant determinants of leptin and ILR whereas the determinants of IAR-index were FRAP (in control

subjects) and HDL-cholesterol (in MS subjects). In all study population BMI was the common determinant of leptin, ILR and IAR-index, and only HDL-cholesterol was the individual determinant of IAR-index.

At this study, we compared the fasting serum concentrations of insulin, leptin, inflammation, and oxidative stress markers in MetS versus healthy elderly subjects. There were significantly higher serum insulin and leptin levels in MS subjects, as compared to controlled subjects. Also, significant negative correlations between serum leptin and fasting glucose were discovered in both study groups (controlled and MetS), whereas the significant inverse correlations of leptin with total cholesterol, LDL-cholesterol, uric acid, and FRAP were pointed out only in the controlled group.

6.4. Discussion

Our results are in line with recent studies that reported similar trends and patterns in elderly patients with type 2 diabetes, showing high leptin levels that were closely linked to obesity and to the length of the disease (67). Our findings are also in accordance with recent studies in adult subjects, which reported a strong association between serum leptin levels with components of MetS, especially in abdominal obesity and insulin resistance. (68,69)

IAR-index in subjects MetS was significantly and positively correlated with uric acid and serum antioxidant capacity (FRAP), and inversely correlated with HDL-cholesterol. Concerning the IAR-index, the most important finding is its significant positive correlation with oxidative damage exerted on proteins (AOPP), pointed out in both control and MS subjects. Also, in both study groups, a trend towards an inverse correlation between ILR and atherogenic index was observed, but this became significant when considering the whole sample study population.

We also aimed to determine the metabolic, oxidative stress, and inflammation predictors of leptin, ILR and IAR-index. By performing multiple linear regression analysis, we found that in MS subjects, only insulin represents a significant determinant of leptin levels, whereas HDL-cholesterol was the significant determinant of IAR-index in both MS subjects, as well as in all study subjects. Moreover, we emphasized the findings that leptin levels were significantly and

positively associated with insulin resistance, and that BMI was the relevant predictor of leptin, ILR, and IAR-index values in all study groups.

We can include our results within findings of a large group of recent studies linking leptin with the pathogenesis of insulin resistance and MS (70,71,72). In morbidly obese subjects, there were significantly higher plasma leptin and leptin-to-adiponectin ratio, as well as, additionally lower concentrations of plasma leptin receptors, which indicate leptin resistance, future risk of insulin resistance, and D2M. (73)

6.5. Conclusions

Our study has shown that in healthy elderly subjects, elevated ILR values have a positive, protective impact on glucose and lipid metabolism, as well as on systemic oxidative stress—assessed as serum antioxidant capacity (FRAP) and protein damage (AOPP). Conversely, lower ILR values reveal perspectives of a higher risk of metabolic impairment, hyperglycemia and dyslipidemia, insulin and leptin-resistance, proatherogenic and prooxidant conditions, endothelial damage, and cardiovascular risk.

Although not completely validated as specific biomarkers for the current exploration of the adipo-insular axis, ILR and IAR-index reflected the biological state of adipose and pancreatic β-cells, and could represent integrated high-potential biomarkers for disease and patient stratification.

7. Clinical Relevance of total serum Antioxidant Status Assay in elderly subjects with Type 2 Diabetes Mellitus

7.1. Introduction and objective

The association between DetM, OS Products and Morbidities and Mortalities in BrCa patients has been demonstrated and we aimed at this study to focus on this relationship.

In this study we compared the clinical relevance - in terms of cardio metabolic risk- of two methods currently used for the analysis of serum total antioxidant status, namely the ferric reducing antioxidant power (FRAP) and the ferric reducing capacity (FRC) and examined their relationships with a marker of serum protein oxidative damage (AOPP), in elderly patients with T2DM.

7.2. Materials and methods

The group of 87 elderly subjects (19 men & 68 women, aged 65-80 years) were enrolled in this study with T2DM.

The levels of glucose, cholesterol, HDL-C, LDL-C, blood urea nitrogen, creatinine, uric acid, FRAP and serum Advanced Oxidation Protein Products (AOPP) were measured. To evaluate the cardiovascular risk, the Atherogenic index (Ai) was calculated by the logarithmically transformed ratio of triglycerides on HDL-cholesterol: Ai = log (TG/HDL-C).

7.3. Results

In fact, normal ageing as well as pre-diabetic and diabetic hyperglycemia are known to be associated with increased production of ROS and oxidative damage to critical molecules in the body: proteins, lipids and nucleic acids (74,75). Nutritional stress, for instance caused by excess high-fat and/or carbohydrate diets, promotes oxidative stress as evidenced by increased lipid peroxidation and protein oxidation products, and by a decreased antioxidant status (76).

The mean values of biomarkers indicating the atherogenic and cardiovascular risk, namely HDL-cholesterol levels were significantly lower whereas the values of fasting glucose, cholesterol, LDL-cholesterol and TG were significantly higher in diabetic subjects compared with the control group. The serum atherogenic index (Ai) which is widely used as CVD risk biochemical marker was significantly higher in T2DM group *versus* control. Non-significant differences between groups were observed for creatinine and blood urea nitrogen, whereas uric acid levels were slightly higher in diabetic patients.

The OS biomarkers were evaluated at systemic level through the assessments of serum total antioxidant capacity (FRAP and FRC) and advanced oxidation protein products (AOPP).

In elderly subjects with type T2DM, significantly higher serum AOPP levels were found out compared with healthy group. Regarding the serum antioxidant status, in all study population, FRAP and FRP were significantly positive correlated with uric acid individual levels

Therefore, the FRAP and FRC values were divided to the individual serum levels of uric acid and expressed also as FRAP-to-uric acid ratio (FRAP/UA) (table 1). FRAP and FRC levels were slightly, non-significantly higher in the T2DM subjects. Moreover, after the additional normalization to uric acid values, the ratios FRAP/UA and FRC/AU remained unchanged

between the studied groups. The study of oxidative stress metabolic determinants which influence the antioxidant status at systemic level revealed important relationships between individual values of FRAP and FRC with biochemical parameters explored in all study population as well as separately, in control subjects and diabetic patients.

Statistical correlation analysis evidenced significant negative associations between the ratios FRAP/UA and FRC/UA with cholesterol and Atherogenic index (Ai) in all study population. A negative relationship between the ratio FRAP/AU and AOPP was pointed out.

Several methods have been developed to assess plasma total antioxidant capacity, some of which being commercialized as reagent kits. Because of their assay principle which is different from case to case, results cannot be comparable when using different methods. Therefore, in the present study we evaluated the serum antioxidant status in healthy elderly subjects and T2DM patients using two analytical methods widely used in different clinical studies - FRAP and FRC. Both method used are based on the same principle – the reduction of iron-complexes followed by a change in color of the reduced Fe^{2+}-complexes. The aim was to correlate these markers with the subject's cardiometabolic risk and with a marker of serum protein oxidative damage (AOPP), in order to investigate, in geriatric subjects, the clinical significance of these biomarkers in monitoring the diabetes progression.

7.4. DISCUSSION

A first observation pointed out was the significant positive correlation of serum antioxidant status expressed as FRAP and FRC with uric acid - a serum compound with low molecular weight acknowledged to fulfill a dual role, explicitly as a hydrosoluble antioxidant and, in the same time, at higher concentrations (hyperuricemia) as pro-inflammatory agent, being regarded as cardiovascular risk marker (77). Moreover, Jansen and Ruskovska (78) also found recently a good correlation of FRAP with uric acid.

We also noticed that in T2DM subjects the FRAP/uric acid ratio displayed better statistically significant correlations with all the biomarkers representative for the cardiometabolic risk: total cholesterol, HDL-cholesterol, triglycerides and atherogenic index, as well as with AOPP which reflect the pro-inflammatory and pro-atherogenic conditions in the endothelial

microenvironment. The decreased FRAP/UA ratio and increased AOPP values could be considered as an early marker of the pathogenesis of diabetes complications in elderly patients.

These new results extend our knowledge on the associations between systemic antioxidant status and oxidative stress markers in elderly subjects with dyslipidemia and insulin resistance. As FRAP and AOPP assays can also be automated and optimized on a clinical auto-analyzer, they are both very suitable for large number of samples and can provide valuable additional information about the antioxidant status, beyond the traditional metabolic parameters.

Our results are in line with a recent study conducted on a sample population composed of 212 T2DM and 208 normal subjects above the age of 40, in which Kharroubi et al. (79)] found significantly higher levels of serum total antioxidant status (TAS) – measured as Trolox equivalents - in type 2 diabetes, and detected several continuous variables associated with the TAS level, namely systolic blood pressure, fasting plasma glucose, glycated hemoglobin, and body mass index. In contrast with these findings Rani and Mythili (79) pointed out a significant decrease in the total antioxidant status among diabetic patients and significant increase in their malondialdehyde levels in comparison to healthy controls.

7.5. Conclusion

Therefore, the present study identified significant associations between total antioxidant status measured using FRAP and FRC assays and increased metabolic risk factors in elderly subjects with type 2 diabetes.

CONCLUSIONS

In the present Thesis we investigated for the first time the network of IGF1/IGF1R, metabolism syndrome metrics and oxidative stress products in breast cancer progression. We studied the expression of IGF1/IGFR1 on breast cancer tissues of women using immunohistochemistry staining and correlate the findings with the levels of IGF1 in serum of the same subjects. Then, when we measured the levels of cholesterol, LDL, HDL, Triglyceride and Glucose with other metrics such as BMI, cigarette smoking and life style as indirect indicators for oxidative stress we found out significant correlation between all these parameters and we compared our results with similar data from international literatures.

1. Our studies showed that the elevation of serum IGF1 levels as well as the overexpression of IGF1/IGF1R in tissues of breast cancer patients are associated with high levels of metabolic syndrome markers as such, LDL cholesterol, triglycerides, glucose, body mass index (BMI) and low levels of HDL.

2. The significant association between IGF1/IGF1R overexpression and Her2 positive/ER Positive support the link of oxidative stress on breast cancer progression.

3. Interestingly, this study revealed remarkable link between malignant subjects and their educational levels which may have reflected in many aspects on their lifestyles such as low physical exercises, diet type and high carbohydrates uptake that increase the risk of breast cancer.

4. We did not find any correlation between IGF1 serum levels and tumor stage or lymph nodes metastases as have been mentioned in previously published reports in breast cancers and other cancer types.

5. We study the biochemical and clinical relevance of new indices of insulin-leptin axis in elderly subjects with metabolic syndrome by creating new insulin-adipogenic indices, namely Insulin-to-Leptin Ratio (ILR) and Insulin-Adipogenic Resistance index (IAR-index). ILR and IARindex reflected the biological state of adipose and pancreatic β-cells and seem to depict the adipoinsular axis status related to metabolic and oxidative stress better than individual markers. Therefore, ILR and IAR-index could represent integrated high-potential biomarkers for disease and patient stratification.

6. We investigated the clinical relevance of serum total antioxidant status and relationships with oxidative stress markers and provided new insights and information regarding the use of specific methods to assess oxidative stress at systemic level with more adapted methods to routine assays, that will enable healthcare professionals – nutritionists, geriatricians, surgeons, to make use of categories of tests for evaluating oxidative stress indicating parameters. These new results extend our knowledge on the associations between systemic antioxidant status and oxidative stress markers in elderly subjects with dyslipidemia and insulin resistance. To gain physicians interest in new techniques of oxidative stress assessment is crucial for the accurate monitoring of diabetes progression.

References

1. PEZ-BERMEJO AL, BUCKWAY CK, DEVI GR, HWA V, PLYMATE SR, OH Y, ROSENFELDRG. Characterization of Insulin-Like Growth Factor-Binding Protein-Related Proteins (IGFBP-rPs) 1, 2, and 3 in Human Prostate Epithelial Cells: Potential Roles for IGFBP-rP1 and 2 in Senescence of the Prostatic Epithelium. Endocrinology 2000;141:4072–4080.
2. Burger AM, Leyland JB, Banerjee K, Spyropoulos DD, Seth AK. Essential roles of IGFBP-3 and IGFBP-rP1 in breast cancer. Eur J Cancer. 2005;41:1515-27.
3. HWA V, YOUNGMAN OH & ROSENFELD RG. The Insulin-Like Growth Factor-Binding Protein (IGFBP) Superfamily. Endocrine Rev 1999;20(6):761–787.
4. Werner H, Sarfstein R, LeRoith D, Bruchim I. Insulin-like Growth Factor 1 Signaling Axis Meets p53 Genome Protection Pathways. J Frontiers Oncology 2016; 6: 159.
5. Simpson A, Petnga W, Macaulay VM, Weyer. CU, Bogenrieder T. Insulin-Like Growth Factor (IGF) Pathway Targeting in Cancer: Role of the IGF Axis and Opportunities for Future Combination Studies. Target Oncol. 2017; 12:571-597.
6. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev. 2002; 23: 824-854.
7. Wee P, Wang Z. Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. J Cancers 2017; 9- 52.
8. Lawlor MA, Alessi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? J Cell Sci. 2001;114(Pt 16):2903-10.
9. Brahmkhatri VP, Prasanna C, Atreya HS. Insulin-Like Growth Factor System in Cancer: Novel Targeted Therapies. Biomed Res Int. 2015; 2015:538019.
10. Christopoulos PF, Msaouel P, Koutsilieris M. The role of the insulin-like growth factor-1 system in breast cancer. Mol Cancer. 2015; 15:14-43.
11. Boyd DB. Insulin and cancer. Integr Cancer Ther. 2003;2:315-29.
12. Choucair A. Crosstalk between IGF-1 and estrogen receptor non-genomic signaling pathway in breast cancer. HAL Id 2018; LYSE1074.
13. NM Probst-Hensch, Probst-Hensch NM, Steiner JHB, Schraml P, et all,. IGFBP2 and IGFBP3 Protein Expressions in Human Breast Cancer: Association with Hormonal Factors and Obesity. Clinical cancer research 2010; 16:1025-1032.

14. Litzenburger BC, Creighton CJ, Tsimelzon A, Chan BT, Hilsenbeck SG, Wang T, et all,. High IGFIR activity in triple-negative breast cancer cell lines and tumorgrafts correlates with sensitivity to anti-IGF-IR therapy. Clin Cancer Res. 2011; 17: 2314–2327.
15. Luque RM, López-Sánchez LM, Villa-Osaba A, et all,. Breast cancer is associated to impaired glucose/insulin homeostasis in premenopausal obese/overweight patients. Oncotarget. 2017;8:8146281474.
16. Goodwin PJ, Ennis M, Pritchard KI, Trudeau ME, Koo J, Madarnas Y, Hartwick W, Hoffman B, Hood N. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. J Clin Oncol. 2002;20:42-51.
17. Kaaks R, Toniolo P, Akhmedkhanov A, Lukanova A, Biessy C, Dechaud H, Rinaldi S, ZeleniuchJacquotte A, Shore RE, Riboli E. Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. J Natl Cancer Inst. 2000;92:1592-600
18. Kozma R, Ahmed S, Best A, Lim L. The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. Mol Cell Biol. 1995;15(4):1942–1952.
19. Nobes CD, Hall A. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell. 1995;81(1):53–62.
20. Wee P, Wang Z. Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. J Cancers 2017; 9- 52.
21. Klement, R.J.; Fink, M.K. Dietary and pharmacological modification of the insulin/IGF-1 system: Exploiting the full repertoire against cancer. Oncogenesis 2016, 5, e193.
22. Koukourakis,M.I.;Giatromanolaki,A.;Simopoulos,C.;Polychronidis,A.;Sivridis,E.Lactatedehydrog enase 5 (LDH5) relates to up-regulated hypoxia inducible factor pathway and metastasis in colorectal cancer. Clin. Exp. Metastasis 2005, 22, 25–30.
23. Kumari S, Badana AK, G MM, G S, Malla R. Reactive Oxygen Species: A Key Constituent in Cancer Survival. Biomark Insights. 2018 ;13:1177271918755391.
24. Turturro F, Friday E, Welbourne T. Hyperglycemia regulates thioredoxin-ROS activity through induction of thioredoxin-interacting protein (TXNIP) in metastatic breast cancer-derived cells MDAMB-231. BMC Cancer. 2007;7:96.

25. De Ita JR, Castilla□Cortázar I, Aguirre GA, Sánchez□Yago C, Santos□Ruiz MO, Guerra□Menéndez L, Martín□Estal I, García□Magariño M, Lara□Díaz VJ, Puche JE, Muñoz U. Altered liver expression of genes involved in lipid and glucose metabolism in mice with partial IGF□1 deficiency: an experimental approach to metabolic syndrome. J Transl Med. 2015;13:326.
26. Aguirre GA, De Ita JR, de la Garza RG, Castilla-Cortazar I. Insulin-like growth factor-1 deficiency and metabolic syndrome. J Transl Med. 2016 Jan 6;14:3.
27. Denley A, Carroll JM, Brierley GV, Cosgrove L, Wallace J, Forbes B, Roberts CT. Differential activation of insulin receptor substrates 1 and 2 by insulin□like growth factor□activated insulin receptors. Mol Cell Biol. 2007;27:3569–77
28. Cohen DH, LeRoith D. Obesity, type 2 diabetes, and cancer: the insulin and IGF connection. Endocr Relat Cancer. 2012;19:F27-45.
29. Arcidiacono B, Iiritano S, Nocera A, Possidente K, Nevolo MT, Ventura V, Foti D, Chiefari E, Brunetti A. Insulin resistance and cancer risk: an overview of the pathogenetic mechanisms. Exp Diabetes Res. 2012;2012:789174.
30. Neuhouser ML, Till C, Kristal A, Goodman P, Hoque A, Platz EA, Hsing AW, Albanes D, Parnes HL, Pollak M. Finasteride modifies the relation between serum C-peptide and prostate cancer risk: results from the Prostate Cancer Prevention Trial. Cancer Prev Res (Phila). 2010;3:279-89.
31. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med. 2010 ;49:1603-16. PMID: 20840865
32. Morgan B, Ezeriņa D, Amoako TNE, Riemer J, Seedorf M, Dick TP. Multiple glutathione disulfide removal pathways mediate cytosolic redox homeostasis. Nat Chem Biol. 2013;9:119-25. 10.1038/NCHEMBIO.1142.
33. Oberley TD, Oxidative damage and cancer. Am J Pathol. 2002;160:403-8. PMID: 11839558
34. https://www.medicalnewstoday.com/articles/324863.php
35. Nita M, Grzybowski A. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Longev. 2016;3164734.
36. Cui H., Kong Y., Zhang H. Oxidative stress, mitochondrial dysfunction, and aging. Journal of Signal Transduction. 2012;2012:13. doi: 10.1155/2012/646354.646354.

37. Evans M. D., Dizdaroglu M., Cooke M. S. Oxidative DNA damage and disease: Induction, repair and significance. Mutation Research. 2004;567(1):1–61. doi: 10.1016/j.mrrev.2003.11.001.
38. Sosa V, Moliné T, Somoza R, Paciucci R, Kondoh H, LLeonart ME. Oxidative stress and cancer: an overview. Ageing Res Rev. 2013;12:376-90. PMID: 23123177
39. Kumari S, Badana AK, Mohan MG, Shailender G, Malla R. Reactive Oxygen Species: A Key Constituent in Cancer Survival. Biomark Insights. 2018; 13: 1177271918755391.
40. Kim J, Kim J, Bae J-S. ROS homeostasis and metabolism: a critical liaison for cancer therapy. Exp Molec Med. 2016;48:e269.
41. Jernström H, Sandberg T, Bågeman E, Borg A, Olsson H. Insulin-like growth factor-1 genotype predicts breast volume after pregnancy and hormonal contraception and is associated with circulating insulin-like growth factor-1 levels: implications for risk of early-onset breast cancer in young women from hereditary breast cancer families. Int J Gynecol Cancer. 2006;16 Suppl 2:497.
42. Liu Y, Zhang J, Huang R, Feng WL, Kong YN, Xu F, Zhao L, Song QK, Li J, Zhang BN, Fan JH, Qiao YL, Xie XM, Zheng S, He JJ, Wang K. Influence of occupation and education level on breast cancer stage at diagnosis, and treatment options in China: A nationwide, multicenter 10-year epidemiological study. Medicine (Baltimore). 2017;96(15):e6641.
43. Lewitt MS, Dent MS, Hall K. The Insulin-Like Growth Factor System in Obesity, Insulin Resistance and Type 2 Diabetes Mellitus. J Clin Med. 2014;3(4):1561-74.
44. Isik B, Ceylan A, Isik R. Oxidative stress in smokers and non-smokers. Inhal Toxicol. 2007;19(9):767-9.
45. Palmer RM,corresponding, Wilson RF, Coward PY, Scott DA. Analysis of circulating insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3) in tobacco smokers and non-smokers. Tob Induc Dis. 2003; 1(1): 12.
46. Tamimi RM , Colditz GA , Wang Y , Collins LC , Hu R , Rosner B , Irie HY , Connolly JL , Schnitt SJ. Expression of IGF1R in normal breast tissue and subsequent risk of breast cancer. Breast Cancer Research and Treatment2011,. 128(1):243-250.
47. http://www.ihcworld.com/_protocols/antibody_protocols/igfir_santa_cruz.htm
48. Sun WY, Yun HY, Song YJ, Kim H, Lee OJ, Nam SJ, Koo JS. Insulin-like growth factor 1 receptor expression in breast cancer tissue and mammographic density. Molecular and Clinical Oncology. 2015, 3(3):572-580.

49. Christopoulos PF, Msaouel P, Koutsilieris M. The role of the insulin-like growth factor-1 system in breast cancer. Molecular Cancer.2015, 14:43]
50. Lange CA , Yee D. Progesterone and breast cancer. Women's Health (London, England)2008, 4(2):151-162.
51. Taketani T, Yamagata Y. Effects of Growth Hormone and Insulin-Like Growth Factor 1 on Progesterone Production in Human Luteinized Granulosa Cells. Fertil Steril.2008; 90 (3):744-8.
52. Li ZJ, Ying XJ,Chen HL , Ye PJ, Chen ZL, Li G, Jiang HF, Liu J, Zhou SZ. Insulin-like growth factor-1 induces lymphangiogenesis and facilitates lymphatic metastasis in colorectal cancer. World Journal of Gastroenterology.2013; 19:7788-7794.
53. Grizzanti J, Lee HG, Camins A, Pallas M, Casadesus G. The therapeutic potential of metabolic hormones in the treatment of age-related cognitive decline and Alzheimer's disease. Nutr Res 2016; 36: 1305-1315.
54. Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. Ann N Y Acad Sci 2010; 1212: E1-E19.
55. Gradinaru D, Margina D, Borsa C, Ionescu C, Ilie M, Costache M, Dinischiotu A, Prada GI. Adiponectin: possible link between metabolic stress and oxidative stress in the elderly. Aging Clin Exp Res 2016; 29: 621-629
56. Guarner V, Rubio-Ruiz ME. Aging, metabolic syndrome and the heart. Aging Dis 2012; 3: 269-279
57. Popa S, Moţa M, Popa A, Moţa E, Serafinceanu C, Guja C, Catrinoiu D, Hâncu N, Lichiardopol R, Bala C, Popa A, Roman G, Radulian G, Timar R, Mihai B. Prevalence of overweight/obesity, abdominal obesity and metabolic syndrome and atypical cardiometabolic phenotypes in the adult Romanian population: PREDATORR study. J Endocrinol Invest 2016; 39: 1045-1053.
58. Fruhbeck G, Gomez-Ambrosi J, Muruzabal FJ, Burrell MA. The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. Am J Physiol Endocrinol Metab 2001; 280: E827-E847.
59. Amitani M, Asakawa A, Amitani H, Inui A. The role of leptin in the control of insulin-glucose axis. Front Neurosci 2013; 7: 51.
60. Pagotto U. Where does insulin resistance start? The brain. Diabetes Care 2009; 32; S174-177.

61. Lam DW, LeRoith D. Metabolic Syndrome. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A (ed). Source Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-2015 May 19.
62. Bremer AA, Jialal I. Adipose tissue dysfunction in nascent metabolic syndrome. J Obes 2013; 393192.
63. Böni-Schnetzler M, Boller S, Debray S, Bouzakri K, Meier DT, Prazak R, Kerr-Conte J, Pattou F, Ehses JA, Schuit FC, Donath MY. Free fatty acids induce a proinflammatory response in islets via the abundantly expressed interleukin-1 receptor I. Endocrinology 2009; 150: 5218-5229 .
64. Ehses JA, Meier DT, Wueest S, Rytka J, Boller S, Wielinga PY, Schraenen A, Lemaire K, Debray S, Van Lommel L, Pospisilik JA, Tschopp O, Schultze SM, Malipiero U, Esterbauer H, Ellingsgaard H, Rütti S, Schuit FC, Lutz TA, Böni-Schnetzler M, Konrad D, Donath MY. Toll-like receptor 2.-deficient mice are protected from insulin resistance and beta cell dysfunction induced by a high-fat diet. Diabetologia 2010; 53: 1795-1806.
65. Jansen EH, Ruskovska T. Comparative analysis of serum (anti)oxidative status parameters in healthy persons. Int J Mol Sci 2013; 14: 6106-6115.
66. Coimbra S, Brandão Proença J, Santos-Silva A, Neuparth MJ. Adiponectin, leptin, and chemerin in elderly patients with type 2 diabetes mellitus: a close linkage with obesity and length of the disease. BioMed Res Int 2014: 701915.
67. López-Jaramillo P, Gómez-Arbeláez D, López-López J, López-López C, Martínez-Ortega J, Gómez-Rodríguez A, Triana-Cubillos S. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. Horm Mol Biol Clin Investig 2014; 18: 37-45.
68. Sudharmadevi MK, Lekshminarayan V, Balakumaran LK, Trivikrama SK. Hyperleptinemia - an independent predictor of metabolic syndrome in the adult population in Kerala, India. Int J Res Med Sci 2016; 4: 3988-3992.
69. Gradinaru D, Borsa C, Ionescu C, Margina D. Advanced oxidative and glycoxidative protein damage markers in the elderly with type 2 diabetes. J Proteomics 2013; 92: 313-322.
70. Yadav A, Jyoti P, Jain SK, Bhattacharjee J. Correlation of adiponectin and leptin with insulin resistance: a pilot study in healthy north Indian population. Indian J Clin Biochem 2011;26:193-196.

71. Al-Hamodi Z, Al-Habori M, Al-Meeri A, Saif-Ali R. Association of adipokines, leptin/adiponectin ratio and C-reactive protein with obesity and type 2 diabetes mellitus. Diabetol Metab Syndr 2014; 6: 99.
72. Iwan-Zietek I, Ruszkowska-Ciastek B, Michalska M, Overskaug E, Goralczyk K, Dabrowiecki S, Rosc D. Association of adiponectin and leptin-to-adiponectin ratio with the function of platelets in morbidly obese patients. J Physiol Pharmacol 2016; 67: 555-561
73. Radak, Z. et al.: Age-associated neurodegeneration and oxidative damage to lipids, proteins and DNA, Mol Aspects Med (2011) Vol. 32, No. 4-6, pp 305-315, ISSN 1872-9452.
74. Tabak, O. et al.: Oxidative lipid, protein, and DNA damage as oxidative stress markers in vascular complications of diabetes mellitus, Clin Invest Med (2011) Vol. 34, No. 3, pp 163-171, ISSN.
75. Newsholme, P. et al.: Molecular mechanisms of ROS production and oxidative stress in diabetes, Biochem J (2016) Vol. 473, No. 24, pp 4527-4550, ISSN 1874-091X
76. Lim, C.S.H & Lim, S.L.: Ferric reducing capacity versus ferric reducing antioxidant power for measuring total antioxidant capacity, Lab Med (2013), Vol. 44, No. 1, pp 51-55, ISSN 0007-5027.
77. Kharroubi, A.T. et al.: Total antioxidant status in type 2 diabetic patients in Palestine, J Diabetes Res (2015), No. 461271, ISSN 2314-6753.
78. Kanbay, M. et al.: Uric acid in metabolic syndrome: from an innocent bystander to a central player, Eur J Intern Med (2016), Vol. 29, pp 3-8, ISSN 0953-6205.
79. Rani, A.J. & Mythili S.V.: Study on the total antioxidant status in relation to oxidative stress in type 2 diabetes mellitus, J Clin Diag Res (2014), Vol. 8, No. 3, pp 108-110, ISSN 0973-709X