*In thankfulness*

*Dedicated*

*to*

*My Parents &*

*to*

*My Brother*

*“It is not that we always thought alike, but that we were always willing to acknowledge the worth of the other’s thoughts.*
*Learning can be and should be satisfying, stimulating, and, yes even fun.“*

***Stanley Robbins***

# Accumulation of Hypoxia–Inducible factor -1 alpha (HIF-1α) Protein and Cyclooxygenase-2 (COX-2) Expression in Human Breast Cancer

**A thesis**

**Submitted to the Medical Research Institute**
**Alexandria University**
**In Partial Fulfillment of the**
**Requirements for the Master Degree**

**in**

**Applied Medical Chemistry**

**By**


## Fatma Suliman Mohammed Abd-EL-Rahman

B.Sc. Chemistry and Biochemistry
Alexandria University, Faculty of science, 2001


**2009**

# Accumulation of Hypoxia–Inducible factor -1 alpha (HIF-1α) Protein and Cyclooxygenase-2 (COX-2) Expression in Human Breast Cancer

**Presented by**

## Fatma Suliman Mohammed Abd-EL-Rahman

B.Sc. Chemistry and Biochemistry
Alexandria University, Faculty of science, 2001

In

**Applied Medical Chemistry**

| **Examiner's Committee** | **Approved** |
|---|---|
| **Prof. Dr. Shehata M. El-Sewedy**<br>Professor of Medical Biochemistry<br>Applied Medical Chemistry Department<br>Medical Research Institute<br>University of Alexandria | ……. |
| **Prof. Dr. Ashraf A.M. Hassan**<br>Professor of Medical Biochemistry<br>Applied Medical Chemistry Department<br>Medical Research Institute<br>University of Alexandria | ……. |
| **Prof. Dr. Mahmoud M.E. Balbaa**<br>Professor of Biochemistry<br>Head of Biochemistry Department<br>Faculty of science<br>University of Alexandria | ……. |
| **Prof. Dr. Mohamed M. Mokhtar**<br>Professor of Human Genetics<br>Human Genetics Department<br>Medical Research Institute<br>University of Alexandria | ……. |

## Advisor's Committee

**Prof. Dr. Shehata M. El-Sewedy** …….
Professor of Medical Biochemistry
Applied Medical Chemistry Department
Medical Research Institute
University of Alexandria

**Prof. Dr. Ashraf A.M. Hassan** …….
Professor of Medical Biochemistry
Applied Medical Chemistry Department
Medical Research Institute
University of Alexandria

**Dr. Wagdy I. Fayed** …….
Assistant Professor of General Surgery
General Surgery Department
Medical Research Institute
University of Alexandria

**Dr. Eman A. El-Abd** …….
Assistant Professor of Radiation Sciences
Radiation Sciences Department
Medical Research Institute
University of Alexandria

# *ACKNOWLEDGMENTS*

First of all, thanks to Allah, creator of all, most Gracious, whose magnificent help was the main factor in accomplishing not only this work but also every affair throughout my life.

I would like to express my sincere thanks to **Prof. Dr. Shehata M. El-Sewedy**, Professor of Medical Biochemistry, Medical Research Institute, University of Alexandria, for his kind help, continuous encouragement, cooperation and advice.

I owe particular gratitude to **Prof. Dr. Ashraf A.M. Hassan**, Professor of Medical Biochemistry, Medical Research Institute, University of Alexandria, for his guidance, encouragement and useful discussion and criticism throughout the preparation of this thesis.

It is my pleasure to thank **Dr. Wagdy I. Fayed**, Assistant Professor of General Surgery, Medical Research Institute, University of Alexandria, for his continuous support and help throughout the medical part of this work.

My deep satisfaction and gratitude go to **Dr. Eman Al-Abd**, Assistant Professor of Radiation Sciences, Medical Research Institute, University of Alexandria for suggesting the research plan, for her guidance throughout the course of the experimental work, and for her help and invaluable advice.

Finally, I would like to extend my thanks to all members of the Applied Medical Chemistry Department, and to the staff of the Molecular Biology Unit, Biotechnology Centre, Medical Research Institute, University of Alexandria, for their co-operation and help, and to everyone shared in making this work possible.

# *ACKNOWLEDGMENTS*

First of all, thanks to Allah, creator of all, most Gracious, whose magnificent help was the main factor in accomplishing not only this work but also every affair throughout my life.

I would like to express my sincere thanks to **Prof. Dr. Shehata M. El-Sewedy**, Professor of Medical Biochemistry, Medical Research Institute, University of Alexandria, for his kind help, continuous encouragement, cooperation and advice.

I owe particular gratitude to **Prof. Dr. Ashraf A.M. Hassan**, Professor of Medical Biochemistry, Medical Research Institute, University of Alexandria, for his guidance, encouragement and useful discussion and criticism throughout the preparation of this thesis.

It is my pleasure to thank **Dr. Wagdy I. Fayed**, Assistant Professor of General Surgery, Medical Research Institute, University of Alexandria, for his continuous support and help throughout the medical part of this work.

My deep satisfaction and gratitude go to **Dr. Eman Al-Abd**, Assistant Professor of Radiation Sciences, Medical Research Institute, University of Alexandria for suggesting the research plan, for her guidance throughout the course of the experimental work, and for her help and invaluable advice.

Finally, I would like to extend my thanks to all members of the Applied Medical Chemistry Department, and to the staff of the Molecular Biology Unit, Biotechnology Centre, Medical Research Institute, University of Alexandria, for their co-operation and help, and to everyone shared in making this work possible.

# *Recommendations*

From our results we can recommend the following points:

- Since COX-2 is not expressed in all breast cancer tumors, and because COX-2 inhibitors have side effects, it is worthy selecting patients who would benefit from COX-2 selective inhibitors. this may provide a rationale for targeting COX-2 in breast cancer therapy
- HIF-1α selective inhibitors also represent new regimen for adjuvant therapy in breast cancer.
- More valuable screening programs for hormonal unbalance might reveal new biomarkers for early diagnosis. In addition, detection of tumors as early as at the angiogenic state might give better chance for the use of COX-2 inhibitors in tumor regression.
- A broad study with large number is mandatory for re-evaluating the relation between LEP and LNM in COX-2 negative breast tumors. Results may reveal the usefulness of LEP inhibitors as a new adjuvant therapy in this group of patients.

## 1.1. Oxygen Homeostasis

Molecular oxygen is essential to almost all forms of life, including human, because it is necessary for the metabolic "burning" of food to produce energy-process known as aerobic metabolism [(1)].

To reach the body cells, where aerobic metabolism takes place, $O_2$ in the air is absorbed through the lungs and into the blood, where it binds to the hemoglobin in red blood cells (RBC). In this form, the $O_2$ distributed through the body, being releasd from hemoglobin and taken up by cells in areas where the $O_2$ level is low [(1)].

Physiological mechanisms that ensure an appropriate level of $O_2$ delivery to tissues have evolved in complex multicellular organisms. Virtually all cells are capable of sensing changes in $O_2$ tension ($PO_2$) and respond adatively when the $O_2$ demands exceeds supply, a codition referred to as hypoxia (Figure 1.1) [(2)].

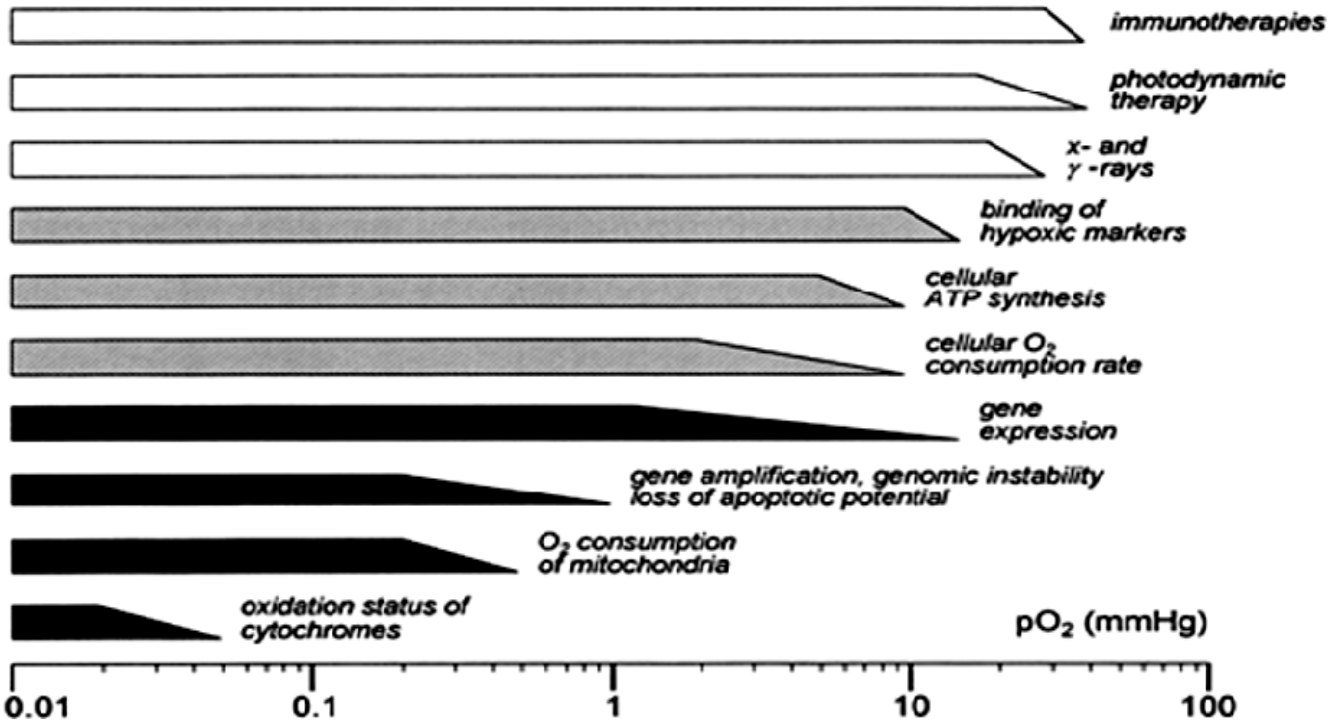


**Figure 1.1.** Adaptive responses to hypoxia [(2)].

## 1.2. Hypoxia

### *A. Definition*

Hypoxia is a Latin word which consists of two parts "hypo" means below and "oxia" means oxygen, so hypoxia refers to inadequate $O_2$ supply to the blood or tissues. This state of oxygen deficiency in the body is sufficient to cause impairments of the body function(s) if prolonged [3].

### *B. Causes*

Hypoxia is caused by either a reduction in the partial pressure of $O_2$ (less than 0.1-1 mm Hg), or inadequate oxygen transport, or the inability of tissues to use $O_2$ [4]. Temporary hypoxia may result from strenuous exercise in which the normal supply of $O_2$ cannot meet the additional requirements of the tissues. The condition disappears once exercise has stopped and breathing has re-oxygenated the tissues. More serious causes include impaired breathing, as a result of lung disorder, ischemia (reduced blood flow to a tissue) due to artery or heart disorder, and severe anaemia in which the $O_2$-carrying capacity of the blood is reduced. Another, rare, cause is carbon monoxide poisoning, which prevents the blood from being adequately oxygenated. In severe cases, any of these more serious causes may led to anoxia (complete absence of $O_2$ in a tissue), which, if prolonged, may cause tissue death [5, 6].

### *C. Occurrence*

Hypoxia can develop as a result of ischemia resulting from hypoperfusion, either as a pathological condition or as a transient physiological (Table1.1) [7].

| Table 1.1 Classification of Hypoxia | | |
|---|---|---|
| **Type** | **Definition** | **Causes** |
| **Hypoxemic Hypoxia** | Reduced availability of atmospheric oxygen "hypoxemic hypoxia" refers to the fact that hypoxia occurs as a consequence of low partial pressure of oxygen in arterial blood | • breathing air at high altitudes (decreased oxygen pressure)<br>• inefficient breathing<br>• obstructed airway<br>• collapsed or damaged lung |
| **Anemic Hypoxia** | Reduced oxygen capacity of blood | • reduced red blood cell count<br>• reduced hemoglobin |
| **Hypemic Hypoxia** | Inability of oxygen to bind to the hemoglobin | • Methemoglobinemia (abnormal version of hemoglobin accumulates in the blood e.g. sickle cell anemia)<br>• Carbon monoxide poisoning (reduced binding capacity of hemoglobin by carbon monoxide) |
| **Ischemic Hypoxia or Stagnant Hypoxia** | Stagnant circulation due to malfunction of the circulatory system resulting in a decrease in blood flow | • shock<br>• cerebral ischemia<br>• ischemic heart disease<br>• heart failure<br>• blocked blood vessel<br>• exposure to extreme hot or cold temperatures |
| **Histotoxic Hypoxia** | Normal delivery of oxygen to tissues, utilization of oxygen by the body tissues is interfered, tissues are unable to metabolize the delivered oxygen (unable to use oxygen) due to disabled oxidative phosphorylation enzymes. | • metabolic poisoning e.g. cyanide<br>• alcohol, narcotics |

### D. In health

Hypoxia occurs in healthy people when they climb to high altitude, where it causes altitude sickness, and the potentially fatal complications of high altitude sickness, high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE). Altitude training causes mild hypoxia to increase the concentration of red blood cells in the body for increased athletic performance. Hypoxia also occurs in healthy individuals when breathing mixtures of gases with low oxygen content in diving [8]. Moderate levels of hypoxia are also known to exist under normal conditions in some normal tissues e.g. skin, esophagus and liver [9].

### E. Pathological disorders

Hypoxia occurs in many common pathological conditions, including some forms of cancer, heart disease, stroke, wounds and bed sores [10]. A common feature of these conditions, is that the blood supply to the hypoxic region delivers less oxygen than required by the affected tissue. Pathological hypoxia also can be classified to generalized hypoxia (affecting the whole body) and tissue hypoxia (affecting a region of the body) [9].

### F. Classification

Hypoxia can be broadly categorized into two types according to its mechanism [11]:

1. Acute or "transient" hypoxia which occurs due to aberrant blood vessels shutting down and reopening. Reperfusion hypoxic tissue with oxygenated blood leads to an increase in free radical concentrations and tissue damage, a process known as 're-oxygenating injury.

2. Chronic or "diffusion-limited" hypoxia which occurs due to limited $O_2$ availability.

Typical adaptation responses generally include changes in the expression of genes encoding molecules that facilitate $O_2$ delivery or by activating metabolic pathways that do not require $O_2$, thus maintaining energy homeostasis. For example, hypobaric hypoxia leads to a classical response characterized by increased RBC mass formation after induction of the erythropoietin (EPO) gene, whose expression is elevated markedly under these conditions (Figure 1.2) [12]. In addition, the vasodilators nitric oxide (NO) and carbon monoxide (CO) are generated by the catalytic activity of inducible NO and heme-oxygenase -1 likewise, up-regulation of vascular endothelial growth factor (VEGF) occurs in response to local vascular hypoxia which leads to vigrous angiogenesis and vasodilation, such as in tumors or during wound healing. Finally, the hypoxia-induced metabolic shift from oxidative phosphorylation to glycolysis as the main source of ATP serves to illustrate another classic homeostatic mechanism in response to $O_2$ deprivation. Hence, increased expression of Glutathione T1 ( GluT1) glucose transporter or glycolytic enzymes such as aldolase A, enolase-1, lactate dehydrogenase A, and phosphoglycerate kinase-1 ensues rapidly after hypoxia to facilitate this metabolic adaptation [13].