INTRODUCTION

Lung cancer is a significant public health problem all-over the world **(Levi et al., 2004).** It is well established that the dominant risk factor for lung cancer is cigarette smoking, causing 90% of all lung cancers in the developed world **(Youlden et al., 2008).**

Lung cancer can be divided into two major histological types; non small cell lung cancer (NSCLC) which accounts for (82 - 85%) of all lung cancer and small cell lung cancer (SCLC) accounting for (15 - 18%) of cases **(Youlden et al., 2008).**

Bronchoscopy is the most important investigation in the diagnosis of lung cancer as it can provide tissue biopsies for direct pathological assessment of the tumor **(Nicholas, 2000).** However, bronchoscopy is considered an invasive technique and is useful only for central lesions while pheripheral lesions are usually not accessible by bronchoscopy **(Murray, 2000).** Tumor markers may also play a role in patient management for many malignancies. The currently available tumor markers for lung cancer include carcinoembryonic antigen, cytokeratin 19 fragment and neuron-specific enolase. However, they are not satisfactory for diagnosis at an early stage owing to their relatively low sensitivity and specificity in detecting the presence of cancer cells. Therefore, there is an urgent need for the

development of novel non-invasive and sensitive diagnostic biomarker, hence improving the disease prognosis through providing better choice of treatment modalities **(Yamabuki et al., 2007).**

Dickkopf -1 (DKK-1) is a 35-KDa protein that contains a single peptide sequence and two cysteine-rich domains. It is the founding member of the Dickkopf family of secreted proteins that includes Dickkopf homolog -1, -2, -3, -4. It is known as a negative regulator of the wnt signalling pathway, which in turn plays an important role in development and regulation of adult stem cell systems. Moreover, a variety of cellular processes are mediated by Wnt signalling, including cell proliferation, differentiation, survival, and apoptosis **(Willert and Jones, 2006).** Hence, loss of regulation of these processes can lead to tumorigenesis **(Gabrielli et al., 2003).**

Many studies have shown over-production of Dickkopf-1 in patients with esophageal cancer, wilms`tumor, hepatoblastoma, hepatocellular carcinoma, breast cancer and multiple myeloma, suggesting that Dickkopf-1 has a potential oncogenic role in these tumors, and may serve as a useful cancer specific antigen **(Patil et al., 2005 and Yamabuki et al., 2007).**

AIM OF THE WORK

The aim of the present study was to assess the clinical significance of serum Dickkopf-1 assay in patients with non small cell lung cancer and its relation to histopathological staging and grading.

I- LUNG CANCER

A- Epidemiology

Worldwide, Lung cancer is the most common cause of cancer death, accounting for more than 1 in 5 cancer deaths, it has one of the lowest survival outcomes of any cancer because over two-thirds of patients are diagnosed at a late stage when curative treatment is not possible. The prevalence of lung cancer is second only to that of prostate cancer in men and third only to that of breast cancer and colorectal cancer in women **(Subramanian et al., 2010).**

In Egypt, the frequency of lung cancer represents about 6.1% in males and 1.3% in females among cancer cases with male to female ratio about 4: 3. The mortality rate 9.7% in males and 5% in females among all cancer deaths **(Persons et al., 2010).**

B- Risk Factors

1- Age and Gender:

The probability of developing lung cancer remains very low until age 39 years in both sexes. It then slowly starts to rise and peaks among those older than 70 years. The risk of developing lung cancer remains higher among men in all age groups after age of 40 years, being 6.74% versus 4.6% among men and women, respectively **(Persons et al., 2010).**

2- Smoking:

Cigarette smoking is the primary cause of lung cancer with smoking-related cancers accounting for approximately 78% of all lung cancer worldwide. Cigarette smoke is a complex mixture of approximately 60-70 carcinogens such as; polycyclic aromatic hydrocarbons (PAHs), heterocyclic hydrocarbons, N-nitrosamines, aromatic amines, N-heterocyclic amines, aldehydes, various organic compounds and inorganic compounds such as hydrazine and some metals **(Pesch et al., 2012).** Other carcinogenic factors in tobacco smoke include polonium-210 which will deposit in the lungs of smokers, emitting alpha radiation which is the most destructive form of ionizing radiations causing chromosomal damage. Cigarette smoke also contains large amounts of free radicals known to induce oxidative damage of the lung **(Persons et al., 2010).** Moreover, cigarette smoke is a strong inflammatory stimulus that induces proinflammatory cytokines as TNF-α and IL-6 which recruits activated macrophages and neutrophils to lung tissue which in turn generate oxidative radicals when exposed to PAHs and aromatic amines. In addition, smoking suppresses the ability of the most hosts to develop the innate immune response to infection. Smoke produced by smoking tobacco is divided into main-stream smoke, which is inhaled and later exhaled by the smoker, and side-stream smoke, which is composed of

smoke emitted directly in the air during burning of a tobacco product and of smoke components that diffuse through the cigarette paper. The excess relative risk among nonsmoking wives of smoking husbands, when compared with nonsmoking wives from nonsmoking husbands, was 24% to 20% in two meta-analyses studies **(Persons et al., 2010).**

3- Environmental Factors:

Air pollution is associated with a variety of respiratory disorders and has long been suspected as a possible pulmonary carcinogen. The major sources of air pollution include transportation, industry, space heating, electric power generation and refuse disposal. The incidence of lung cancer is slightly higher in urban than in rural dwellers, this may reflect differences in atmospheric pollution. In some cities, air pollution can increase the risk of lung cancer; this risk is far less than that of smoking **(Dvm et al., 2011).**

In addition to air pollution, radon gas is the second leading cause of lung cancer in the United States. It is a naturally reactive but chemically inert gas found ubiquitously in the environment, emanates as a toxic gas from the soil and from building material of terrestrial origin, such as stone, bricks, and concrete. The carcinogenicity of radon is attributable mainly to its short-lived, radioactive, alpha emitting atoms, polonium that

by its virtue of its high energy and mass can cause damage of the DNA of the cells of the respiratory epithelium **(Ferlay et al., 2010).**

Overall estimates have been made that radon may contribute to 9% of all lung cancers, and that the exposure to radon and smoking are at least additive factors **(Ferlay et al., 2010).**

4- Occupational Exposure:

The inhalation of asbestos fibers causes serious illness including lung cancer and mesothelioma. The evidence for lung cancer induction by occupational exposure to metals such as beryllium, chromium, nickel and arsenic is convincing and well documented**.** Workers who are exposed to tar and soot, such as coke oven workers in concentrations that exceed those present in urban air are at increased risk for lung cancer **(Jawhari et al., 2012).**

Exposure to asbestos is widespread in developing countries among people who were occupationally exposed to chrysolite and may be significant etiologic factors for lung cancer. Moreover, asbestos can be found naturally in the air outdoors and in some drinkable water, including water of natural source **(Jawhari et al., 2012).**

5- Diet and Nutrition:

Iso-thiocyanates are naturally occurring small molecules that are formed from glucosinolate precursors of cruciferous vegetables. Iso-thiocyanates, display anticarcinogenic activity because they reduce activation of carcinogens and increase their detoxification. A study done by **Kim and Kim (2011)** showed that they exhibit anti-tumor activity by affecting multiple pathways including apoptosis, oxidative stress, and cell cycle progression.

Some reports have indicated that a diet low in fruits and vegetables may increase the chance of getting cancer if the patient was exposed to tobacco smoke **(Kim and Kim, 2011).**

6- Benign Lung Diseases:

Lung cancer incidence has been reported to be higher in patients with benign lung diseases such as tuberculosis, chronic bronchitis, chronic obstructive pulmonary disease (COPD) and silicosis, especially when associated with chronic cigarette smoking that retards clearance of foreign particles and respiratory tract secretions leading to structural and functional broncho-pulmonary damage, which results in sustained exposure of the mucosa to the carcinogenic products of tobacco consumption **(Siegel et al., 2014).**

Molecular genetic studies have shown the acquisition by lung cancer cells of a number of genetic lesions including activation of proto-oncogenes **(table 1)** and a number of inactivated tumor suppressor genes **(table 2).**

Table (1): Activated Proto-oncogenes in Lung Cancer.

Oncogene	SCLC	NSCLC
Ki-ras	0	30%-50% of adenocarcinoma (activating mutation)
H-ras	0	Rare mutation, over-expression occurs
N-ras	0	Rare mutation, over-expression occurs
Myc (c, L, N)	Majority	Gene amplification and over-expression
Her2/neu	-	30% (over-expression)
c-kit	Over expression	-
bel-2	-	Over-expression
Cyclin D1 (prad)	-	Over-expression

(SCLC= Small cell lung cancer, NSCLC= Non small cell lung cancer, ras= homologus to rat sarcoma virus, myc = myelocytoma gene, Her2= human epidermal growth factor2, bel-2=bla gene)

(Oxnard et al., 2013)

Table (2): Inactivated Tumor Supressor Genes in Lung Cancer.

Chromosomal deletion	Tumor suppressor genes inactivated
3p14-25	Unknown, probably multiple (50% NSCLC, 90% SCLC).
5q	APC
9p21	CDKN2A, CDKN2B, possibly others (100% NSCLC, not reported in SCLC)
13q14	Rb (100% SCLC, 20% NSCLC)
17p13	TP53 (90% SCLC, 60% NSCLC)

(SCLC= Small cell lung cancer, APC = adenomatous polyposis coli gene, CDKN =cyclin dependant kinase inhibitor gene, Rb = retinoblastoma gene, TP53=tumor suppressor protein 53).

(Oxnard et al., 2013)

7- Radiation Therapy:

People who have received radiation therapy to the chest for cancer are at higher risk for lung cancer. Typical patients are those treated for Hodgkin disease or women who get radiation after a mastectomy for breast cancer (**Henson et al., 2012).**

8- Viruses:

Viral causation of lung cancer has been intermittently considered. There is evidence supporting human papilloma virus, John Cunningham virus **(**JCV) **(Tan et al., 2015**), Simian

virus 40 (SV40) and Cytomegalovirus (CMV) **(Oser et al., 2015)** as possibly contributing to lung cancer.

9- Socioeconomic Status:

Lung cancer is more likely to occur in the poor and less educated pattern that is observed in many countries world-wide. Socioeconomic status is associated with a constellation of interacting determinants of lung cancer risk, such as smoking, diet and exposure to inhaled carcinogens in the workplace and general environment. Lower socioeconomic status is associated with an unfavorable profile for all of these factors **(Aldrich et al., 2013).**

C- Pathogenesis of Lung Cancer:

Lung carcinogenesis is a multi-step process resulting from the accumulation of altered molecules generated from genetic and epigenetic abnormalities of genes which are involved in cell cycle, apoptosis, repair, differentiation, cell migration controls and angiogenesis. Uncontrolled cell growth is derived from either oncogene activation or tumor suppressor gene inactivation **(Vollmer et al., 2010**).

1- Mechanism of Malignant Transformation Processes:

Transformation of a normal phenotype into a malignant phenotype requires accumulation of multiple genetic changes

with each step resulting in some forms of growth and/or cellular survival advantage. Critical alterations involved in this multi-step process are loss of tumor suppressor gene (TSG_s), proto-oncogene activation, and deregulation of apoptosis and telomerase control, sustained angiogenesis and tissue invasion. Additionally, malignant transformation is characterized by genetic instability at a chromosomal level as chromosomal translocations and micro-satellite instability **(Brewer et al., 2012).**

Lung cancer processes is characterized by cumulative alterations in key molecules involved in the cell cycle, signaling and angiogenesis pathways. Most lung cancer patients demonstrate chromosomal abnormalities at the site of tumor suppressor genes or have mutations in known oncogenes. Loss of hetrozygosity is common **(Vollmer et al., 2010).**

Tumor cells that produce a growth factor and express its receptor may show self-stimulatory or autocrine growth. Cells that are regulated by an autocrine loop have several features. They secrete a biologically active growth factor and demonstrate increased proliferation in response to that factor. Antibodies that bind specifically to the growth factor will inhibit cell growth. Growth factors may act to stimulate growth in adjacent cells in a paracrine manner. Interaction of ligand and receptor in the cytoplasm of the cell may form an internal autocrine loop.

Amplification of oncogenes and inactivation of tumor suppressor genes have been identified in NSCLC. The most important abnormalities detected are mutations involving the ras family of oncogenes. The ras oncogene family has 3 members: H-ras, K-ras, and N-ras. These genes encode a protein on the inner surface of the cell membrane with guanosine triphosphatase activity and may be involved in signal transduction. Studies in humans suggest that ras activation contributes to tumor progression in persons with lung cancer. The ras gene mutations occur almost exclusively in adenocarcinomas and are found in 30% of such cases (**Brewer et al., 2012) (Figure 1).**

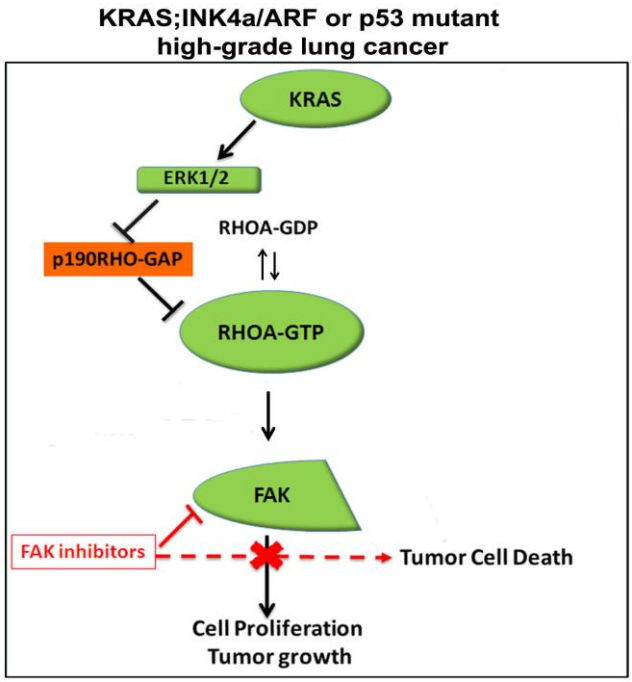

Fig. (1): Ras gene mutation in lung cancer **(Brewer et al., 2012).**

2- Angiogenesis and Tissue Invasion:

Tumors beyond 2-3 mm^3 in size require functional vasculature to sustain growth and metastasize. The amount of microvessels in tumors or microvessel density correlates with metastatic potential and prognosis. Angiogenesis is essential for tumor growth and metastasis, controlling tumor-associated angiogenesis is a promising tactic in limiting cancer progression. The tumor microenvironment comprises numerous signaling molecules and pathways that influence the angiogenic response. Regulation of angiogenesis occurs through a balance of angiogenic and angiostatic factors. In response to metabolic demands, e.g. hypoxia, tumor cells promote angiogenesis by producing growth factors that lead to the formation of new blood vessels from pre-existing vasculature **(Semenza, 2012).**

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor with a selective mitogenic effect on vascular endothelial cells. There are five VEGF isoforms that are generated from one gene. The expression of VEGF is augmented in response to hypoxia but also by activation of the RAS oncogene. VEGF is associated with a poor prognosis and presence of lymph node metastasis. It appeared to be associated with high micro-vessel density and its expression increase in high-grade lesions. This indicates that VEGF might be involved early in the carcinogenic process **(Semenza, 2012).**

3- Metastases:

The major steps in the pathogenesis of metastasis are growth of neoplastic cells, extensive angiogenesis, and local invasion of the host stroma by some tumor cells and penetration of thin-walled venules by tumor cells. These steps in turn provide a common pathway for tumor cell entry into the circulation, embolization and detachment of small tumor cell aggregates, arrest of small tumor cell aggregates in the capillary beds of organs, either by adhering to capillary endothelial cells or by adhering to subendothelial basement membrane, proliferation within the vessel or extravasation of tumor cells and proliferation within the organ parenchyma to complete finally the metastatic process. To continue growing, the micrometastases develop a vascular network via angiogenesis and continue to evade the host immune system. The metastatic cells can invade, penetrate blood vessels, and enter the circulation to produce additional metastasis, a process known as metastasis of metastasis **(Semenza, 2012).**

D- Classification of Lung Cancer:

1- Histopathological Classification:

Lung cancer is divided into two main histological groups. 85% of lung cancers are non small cell lung cancer (NSCLC) and 15% are small cell lung cancer (SCLC). The NSCLC group