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Original Research Article

Immunohitochemical Study of Anaplastic Lymphoma Kinase (ALK) Overexpression in Non-Small Cell Lung Carcinoma (NSCLC) and its Clinicopathological Correlation

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Abstract

Lung cancer is the most common cancer in men while is third highest incidence among women and is second after breast cancer in mortality. Most cases occur in older people in age 65 or older, while less than 2% are younger than 45. The pathogenesis of NSCLC is complex and developed in multistep process in which several gene mutations will occur and coordinate with each other in genotyping and phenotyping outcome. Non-small cell lung carcinomas (NSCLC) which represent 85% of all lung cancers, it represents the most common type, usually grows and spreads more slowly than other types, histologically is divided into Adenocarcinoma (ADC), Squamous cell carcinoma (SCC) and large cell carcinoma (LCC). Its incidence and mortality rates vary markedly around the world. The aim of this study was to assess Anaplastic Lymphoma Kinase (ALK) over expression in Non-small cell lung carcinomas (NSCLC) and its correlation with type, grade and stage of the tumor.

Forty cases of NSCLC was included in this study, collected randomly from the period of February 2016- April 2017 from Al-Harirey Teaching Hospital in Baghdad and Al-Sadder Teaching Hospital in Al-Najaf governate. The (40) patients (22 male and 18 females) of NSCLC, their median age 58.3 years range from (34-80) years. A group of 12 cases with benign brain tissue were included as a control group. A manual Envision procedure was used in the imunohistochemical analysis (Dako Cytomation Copenhagen, Denmark). ALK over expression was positive in (12.5%) of NSCLC, ALK immunohistochemical staining was more in ADC than SCC and its not correlated with grade and stage of NSCLC. These finding support the role of ALK in carcinogenesis of ADC and less commonly SCC of the lung.

Key Words: NSCLC, a manual Envision procedure, ALK.

الخلاصة:

يشكل سرطان الرئة ذو الخلايا السرطانية الغير صغيره نسبة ٨٥% من سرطان الرئة وهو اكثر الانواع شهرة. وعادة يكون ذو نمو وانتشار بطيء قياسا بالأنواع الاخرى. نسيجيا يقسم الى ثلاثة انواع الحرشفي والغدي الطلائي ونوع الخلايا الكبيرة. نسبة حدوث سرطان الرئة ذو الخلايا السرطانية الغير صغيره و نسبة الموت المتسبب منه في زيادة مستمرة في كل مناطق العالم حيث يعتبر سرطان الرئة بصوره عامه الاكثر شيوعا عن الرجال بينما يحتل المرتبة الثالثة بالنسبة للنساء. ان نسبة الاصابة بهذا المرض تزداد بازدياد التقدم بالعمر وليزداد ظهوره في سن الخامسة الستين او اكبر بينما تشكل نسبة الاصابة بهذا المرض ٢% لمن هم اصغر من سن خمسة واربعون عاما . يعتبر سرطان الرئة ذو الخلايا السرطانية الغير صغيره معقد من خلال حدوثه ونشوئه و يتضمن عدة خطوات متسلسلة لطفرات جينية تعمل معا لتتسبب بظهور خلل جيني و مظهري .تتضمن الدراسة ٤٠ حالة خلال الفترة شباط ٢٠١٦ الى شهر نيسان ٢٠١٧ من مستشفى الحريري في بغداد ومستشفى الصدر التعليمي في النجف. هذه ٤٠ حالة تقسم الى (٢٢ حالة ذكر و١٨ حالة انثى٩) متوسط العمر (٥٨،٣) سنة. تم أخذ ١٢ حالة من النسيج الدماغي كمجموعة مقارنة لعامل ALK وتم استخدام طريقة الـ Envision.

النتائج: نسبة ALK (12.5%) في سرطان الرئة ذو الخلايا السرطانية الغير صغيره ونسبته اعلى في الغدي الطلائي من النوع الحرشفي .

الاستنتاجات: تظهر من النتائج اعلاه دور ALK في نشوء سرطان الرئة ذو الخلايا السرطانية الغير صغيرة من نوع الغدي الطلائي أعلى النوع الحرشفي.

Introduction

NSCLC which represent 85% of all lung cancers. It's the most common type, usually grows and spreads more slowly than other types, histologically is divided into Adenocarcinoma (ADC), Squamous cell carcinoma (SCC) and large cell carcinoma (LCC) [1]. Its incidence and mortality rates vary markedly around the world. Lung cancer is the most common cancer in men while is third highest incidence among women and is second after breast

cancer in mortality its prevalence rates seem to be higher in the developed and industrialized countries like America and Northern Europe than in the developing countries of the world [2]. Most cases occur in older people in age 65 or older, while less than 2% are younger than 45. In Iraq and according to the Iraqi cancer registry 2011, lung cancer was occupied the second of ten common cancers in Iraq (1812 new case was recorded 8.94%), its classified as the first famous cancer in male (1380 case with percentage 14.76%) and seventh famous type of cancer in female (432 case 3.95%) [3]. The pathogenesis of NSCLC is complex and developed in multi steps process in which several gene mutations will occur and coordinate with each other in genotyping and phenotyping outcome [4]. Tobacco smoking is the primary and the most important etiologic risk factor for lung cancer, approximately 85% of cases of lung cancer are directly attributable to smoking habits [5], other risk factor as Radon [6] and air Pollution particularly air with a high concentration of fine particulates such as sulfate aerosols or nitrogen dioxide. Additionally, there is some evidence that exposure to aluminum, cadmium, chromium, beryllium, iron, nickel, arsenic, hematite, coal, diesel, radiation, silica and toxic dust may cause lung cancer [7]. SCC is more common in men 44% than in women 25% usually associated with a history of smoking than other types of NSCLC. It most often arises centrally in larger bronchi, often preceded for years by squamous-cell metaplasia and dysplasia in the respiratory epithelium of the bronchi, which later transforms to carcinoma in situ and its frequently associated with central necrosis and calcification, Microscopically: there is keratinization in the form of 'keratin pearls' and/or intercellular bridges. SCC are graded into well, moderately, and poorly differentiated depend on the amount of keratin present and pleomorphism of cells as grade of pleomorphism [8].

ADC represents the most common type of lung cancer in females, nonsmokers and in people under the age of 45 among Asians, it's usually begins in the peripheral parts of the lungs, cavitation is unusual and about 65% of the cases are located peripherally, different histologic subtypes in lung adenocarcinomas include lepidic, acinar, papillary, micro papillary and solid subtypes [9].

(Undifferentiated) large cell carcinoma: These are heterogeneous group of undifferentiated malignant neoplasms that lack the cytological and architectural features of small cell carcinoma and glandular or squamous differentiation. Its incidence 5-10% of all lung cancers and the risk of large cell lung carcinoma increases with a previous history of tobacco smoking. LCC is considered as a type of NSCLC because it originates from epithelial cells of the lung. These tumor consists of pleomorphic malignant epithelial tumors without any proof of either squamous or glandular differentiation [10].

NSCLC has been graded into well, moderately and poorly differentiated [11]. (TNM) classification for lung cancer was published in September 2009 and enacted in January 2010. The revision was novel in that the changes were based entirely on the proposals of the International Association for the Study of Lung Cancer (IASLC) International Staging Project [12].

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) belonging to the insulin receptor superfamily, the ALK gene is located on the short arm of chromosome 2 and was first identified as an oncogene activated by chromosomal translocation t(2;5)(p23;q35) in anaplastic large cell lymphoma (ALCL) patients ALK, activation of ALK derived from chromosomal rearrangements, mutations, or amplification of the ALK [13]. ALK is normally expressed only in the nervous system, usually expressed in specific regions such as the thalamus and midbrain, suggesting that ALK plays an important role in the development and maintenance of the central and peripheral nervous systems [14], ALK gene has been linked to tumorigenesis and progression of certain cancers such as non-small cell lung carcinoma (NSCLC), breast cancer [15], and neuroblastoma [16]. The human ALK gene encodes a 176 kDa protein, the ALK receptor is a single pass trans membrane protein that consists of an extracellular region of 1.030 amino acids [17], containing an N-terminal

signal peptide, two meprin, A-5 protein, receptor protein tyrosine phosphatase mu (MAM) domains separated by a low-density lipoprotein class A (LDL-A) domain, and a glycine-rich region proximal to the transmembrane domain that connects the extracellular region with the intracellular region. The MAM domains of this receptor consist of approximately 160 and are thought to participate in cell–cell interactions, [18] whereas the function of the LDL-A domain is still unknown. The kinase domain contains three autophosphorylation sites in tyrosine residues 1278, 1282, and 1283, known as the YXXXYY motif, whose phosphorylation regulates the kinase activity of ALK (figure 1A) [19].

Ligands of Anaplastic Lymphoma Kinase: midkine (MK) and pleiotrophin (PTN) are growth factors considered as putative endogenous ALK ligands capable of acting as autocrine/ paracrine signaling molecules [20], MK and PTN are expressed during development of the nervous system and are highly expressed in some cancers where they act as angiogenic factors that have a role invasion and metastasis [21]. MK is a heparin-binding growth factor with a molecular weight of 13 kDa that regulates development of lung, kidney, bone, and nervous systems.MK stimulates ALK phosphorylation and activates phosphatidyl-inositol 3-kinase (PI3K) and MAP kinase signal transduction PTN is a 18 kDa protein that acts as a growth factor, regulating neurite outgrowth and proliferation of fibroblasts and endothelial cells [22].

Materials and Methods

This study was carried out in the Department of Pathology in the College of Medicine-University of Kufa, it included forty paraffin embedded samples (22 male and 18 females) cases with NSCLC, (20) cases of ADC and (20) cases SCC were collected randomly from the period of February 2016- April 2017 from Al Harirey Teaching Hospital in Baghdad and Al Sadder Teaching Hospital in Al Najaf governments.

The clinical information were collected including age, sex, histological type, stage and grade of the NSCLC from the clinical reports of the hospital, the median age 58.3 years range from (34-80) years. The cases reviewed by two pathologist of classification according to WHO classification system of NSCLC classified into (12) cases stage T1, (10) cases stage T2, (14) cases stage T3 and (4) cases stage T4, the cases according to the grade of the tumor were classified into three grades as (14) cases grade I, (20) cases grade II and (6) cases grade III. Tissue sections of 5-Mm thickness from formalin-fixed, paraffin-embedded blocks were taken for the Envision procedure was used for imunohistochemical detection of ALK.

The criterion for positive reaction was dark brown staining precipitate at cytoplasmic staining pattern. The immunostaining was calculated as the percentage of immune-reactive cells per total number of malignant cells. Each sample was scanned for at least five fields with a high power magnification. Intensity scoring of ALK protein over-xpression [23].

0 No staining
+1 Faint cytoplasmic staining (weak)
+2 Moderate smooth cytoplasmic staining (moderate)
+3 Intense granular cytoplasmic staining in ≥10 % of tumor cells (strong).

Results

In the study group, ALK overexpression was reported in 5 (12.5%) out of 40 cases, 35 (87.5%) cases were negative, ALK overexpression was reported in only one case (5%) of SCC out of (20), 4 (20%) out of 20 of ADC. There is no significant difference between the types of NSCLC in relation to ALK protein (P value = 0.1515) and there is no correlation between the types of NSCLC and ALK protein overexpression (r = -0.227) (Table 1).

The intensity of ALK overexpression in relation to the type of the tumor revealed that, positive case of SCC was score +1 and all the cases of ADC were score +2. There is no correlation and no significant difference between different tumor types and the intensity of ALK overexpression (P>0.05) table (2).

In our study according to the grade of distribution, ALK overexpression in relation to grade of tumor revealed that positive ALK was reported in 1 (7.1%) of grade I, 4(20%) of grade II, and no positive case was reported of grade III, with no significant difference between the detection rate of ALK immunostaining and grade of tumor in relation to the number of the cases but there is difference in the percentage of the positive cases between grade I and II(p=0.494)(r=-0.111) (Table 1). The intensity of immunostaining of ALK protein was assessed in relation to the grade of tumor, score +1 reported in 2 cases, 3 cases on score +2 and no case on score +3 so there is no significant difference between the intensity of ALK and the grade of tumor (P >0.05) (Table 2).

According to size (T) of tumor, ALK overexpression was positive in ALK overexpression was reported in one case out of (12) of T1 represented (8.3%), one case (10%) out of (10) cases of T2, 3(21.42%) out of (14) of T3 and no positive case of T4 was reported. There is no significant difference between ALK expression and size (T) of tumor in relation to the number of the cases but there is difference in the percentage of the positive cases (p=0.727)(r= -.057) table (1). The intensity of ALK overexpression in relation to the tumor stage revealed that, 2 case of score +1 and 3 cases with score +2 and no cases with score +3 so there is no correlation and no significant difference between different tumor stages and the intensity of ALK overexpression (P>0.05) table (2).

Table (1): ALK overexpression in relation to type of tissue, grade and stage of NSCLC.

Parameter	Positive	Negative	Total	P value
Tumor type				
ADC	4 (20%)	16 (80%)	20(50%)	P=0.01515 NS
SCC	1 (5%)	19 (95%)	20(50%)	
Tumor Grade				
Grade I	1(7.1%)	13(92.9%)	14(35%)	P=.0494
Grade II	4(20%)	16(80%)	20(50%)	
Grade III	0	6(100%)	6(15%)	
Tumor size (T)				
T1	1(8.3%)	11(91.7%)	12(30%)	P=0.727 NS
T2	1(10%)	9(90%)	10(25%)	
T3	3(21.42%)	11(78.57%)	14(35%)	
T4	0	4(100%)	4(10%)	

Table 2: The score of ALK overexpression in relation to type, grade and stage immune-staining in NSCLC.

Parameter	Total number of cases	ALK Intensity (score)			
		Score0	Score+ 1	Score+ 2	Score +3
Tumor type					
ADC	20	0	1	3	0
SCC	20	0	1	0	0
Tumor size					
T1	12	0	0	0	0
T2	10	0	1	1	0
T3	14	0	1	2	0
T4	4	0	0	0	0
Tumor grade					
GI	14		1	0	0
GII	20		0	3	0
GIII	6		1	0	0

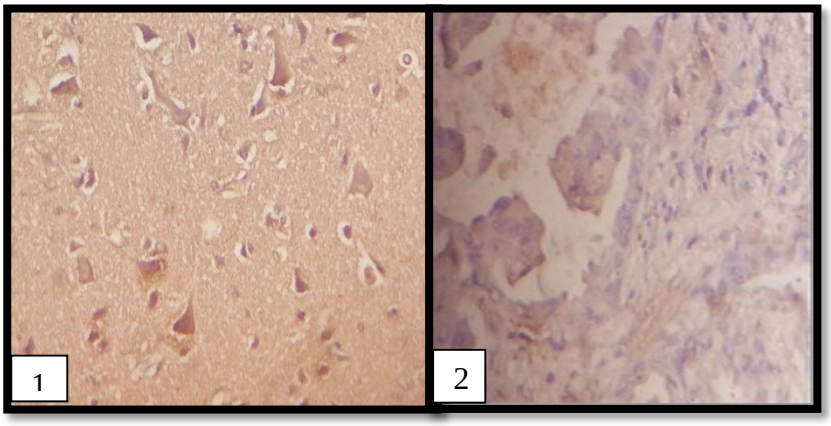

Figure (1): Normal brain tissue positive for ALK with cytoplasmic expression (positive control).
Figure (2): Grade II ADC positive for ALK with cytoplasmic expression score 2, X40.

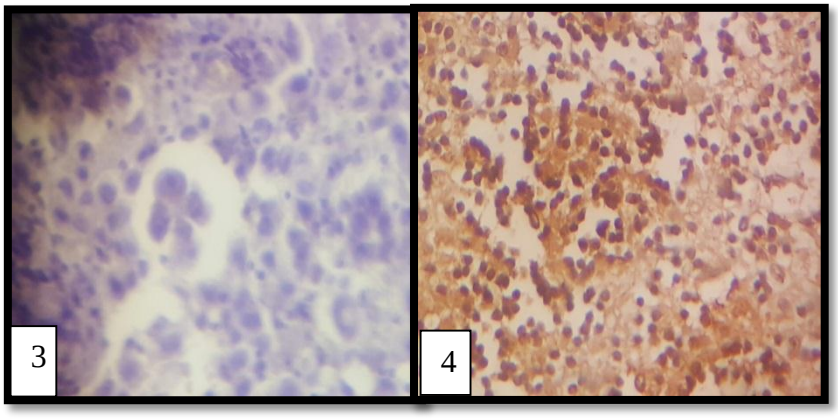

Figure (3): Grade II SCC negative for ALK with cytoplasmic expression X40.
Figure (4): Grade I ADC negative for ALK with cytoplasmic expression X40.

Discussion:

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) belonging to the insulin receptor superfamily ALK gene has been linked to tumorigenesis play an important role in regulating different types of cellular processes, such as proliferation, differentiation, survival and malignant transformation, and progression of certain cancers such as non-small cell lung carcinoma

(NSCLC) [24], ALK overexpression in NSCLC is variable, ranging from 0.4-15% [25]. These variations in reported rates of ALK are due to differences in applied scoring criteria for the assessment of ALK overexpression, or could be attributed to tumor heterogeneity, variability in staining protocols, small biopsies containing minimal tumor cells, and sometime, Immunohisto-chemical staining can be hindered by poor fixation [26].

In our study, positive ALK overexpression Imunohistochemical staining was found in 12.5% (5 out of total 40 cases) of the study group of NSCLC, ALK detection rate more in in lung Adenocarcinoma (ADC) (4 out of 20 cases) than SCC (1 out of 20 cases) There is no significant difference between the types of NSCLC in relation to ALK protein (P = 0.015) and there is no correlation between the types of NSCLC and ALK protein overexpression (r=-0.227) (Table 1).

This finding agrees with that reported by Jiandong Wang *et al* [27] who found that 3 out of 207 (1.4%) cases of lung SCC were ALK positive detected by IHC staining.

This data was documented by other research agrees with TO KF *et al*. [28] who found that found (22) out of (373) lung ADC (5.9%) were positive for ALK immune-reactivity, ALK-positive tumor cells demonstrated strong and diffuse granular staining in the cytoplasm. Regarding the intensity of ALK overexpression in NSCLC revealed that; one case out of 40 were score +1 (weak), 3 cases with score +2 (moderate) one case with score +3 (strong). There is no significant difference between the different histological types in relation to intensity of ALK overexpression P=0.01515, this agree with Yuan Li *et al* [29] that who found most cases (+2, +3).

In our study according to the grade distribution, there was no significant difference between the detection rate of ALK immunostaining and grade of tumor in relation to the number of the cases but there is difference in the percentage of the positive cases between grade I (7.1%) and grade II(20%) (p.494) (r=-0.111). Our results disagree with Raheleh Roudi *et al* [30], who made a retrospective study reviewed 140 samples of NSCLC, including 64 (46%) SCC, 62 (44%) ADC, and14 (10%) LCC for expression of ALK using immuno-histochemistry and its correlated with clinicopathological parameters, the highest level of ALK expression was found in ADC cases with significant differences between SCC with ADC and LCC samples (P< 0.001), with poor differentiation and high nuclear grade (P=0.005 and P=0.005, respectively).

The immunohistochemical analysis of the results revealed that ALK overexpression in the presented NSCLC cases was noticed in 8.3% of T1, 10% of T2, 21.42% of T3, and none of T4 (Table 2).

From these readings of ALK overexpression, it looks that stage T3 more ALK immunostaining will be noticed so there is significant difference between (T1, T3) (p= 0.727) (Table 2).

These findings agreed with Raheleh Roudi *et al* [30], who found that ALK overexpression was correlated with more aggressive clinical behavior. In conclusion, ALK overexpression is more and variable in ADC than SCC with more aggressive clinical course but without significant relationships between total ALK overexpression and grade and stage of NSCLC.

These findings further support the role of ALK in the carcinogenesis of NSCLC especially ADC regarding behavior and aggressiveness of tumor and thus ALK could be considered as bad prognostic parameter in ADC in which we needed larger study focusing on immunohistochemical expression of ALK in ADC and SCC and further concurrent genetic DNA analysis in different histological types of NSCLC.

Conclusion:

ALK immunohistochemical detection rate are more in ADC than SCC, and may be useful in selecting patients for adjuvant therapy**.**

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