RESEARCH ARTICLE

Association of High LDH and Low Glucose Levels in Pleural Space with HER2 Expression in Non-Small Cell Lung Cancer

Bijan Ziaian[1], Ali Saberi[1], Mohammad Ali Ghayyoumi[2], Akbar Safaei[3], Abbas Ghaderi[4], Zahra Mojtahedi[4]*

Abstract

Background: Evidence shows direct link of HER2 to increased glycolysis and over production of lactate dehydrogenase (LDH). HER2 overexpression, high LDH and low glucose pleural levels are associated with poor prognosis in lung cancer. Here, their relationships were investigated. Materials and Methods: HER2 positivity was studied using immunohistochemistry in non-small cell lung cancer. Glucose and LDH levels were measured using commercial colorimetric kits. Results: Of 42 patients (29 adenocarcinoma and 13 squamous cell carcinoma), 28 (66.7%) were HER2-negative, 14 (33.3%) were HER2- positive, including 9 (21.4%) weakly stained (1+) and 5 (11.9%) moderately stained (2+) samples. The relationship between HER2 and glucose and LDH levels were tested in 20 newly diagnosed lung cancer patients who had simultaneous pleural and serum samples. Pleural and serum LDH levels were increased, and pleural glucose levels were decreased with the scale of HER2 positivity, and that the difference in glucose levels between HER2-negative group and HER2-positive patients scored at 2+ reached statistical significance (p=0.02). This latter group all had pleural glucose levels below 40 mg/dl. Conclusions: For the first time, we showed a significant association between low pleural glucose level and overexpression of HER2 in lung cancer. Further investigations are warranted to disclose the association of HER2 with low pleural glucose levels in other populations, with a larger sample size, in malignant pleural effusions caused by other types of cancer, and finally to assess employment as a screening tool for finding HER2-positive cases of lung cancer.

Keywords: Glucose - HER2 - LDH - lung cancer - malignant pleural effusion

Asian Pac J Cancer Prev, **15 (4)**, 1617-1620

Introduction

Lung cancer is one of the leading causes of cancer worldwide (Pirker and Minar, 2010; Masoompour et al., 2011). The initial presentation in approximately 15% of the patients is malignant pleural effusion (MPE), which is defined by the presence of cancer cells in the pleural space. Lung cancer patients with MPE have a very poor survival (Heffner and Klein, 2008). Even, the survival of this group of the patients is predicted to worsen if certain biochemical characteristics of MPE are changed (Sanchez-Armengol and Rodriguez-Panadero, 1993; Bielsa et al., 2008).

There is a general agreement on the association of low glucose and high lactate dehydrogenase (LDH) pleural levels with poor prognosis in MPE (Ernam et al., 2005). For example, Sanchez-Annengol and Rodriguez-Panadero (1993), found that pleural glucose level less than 60 mg/dl is a sign for a significant shorter survival in cancer patients than in the group with higher glucose. Bielsa et al. (2008), reported that the survival of MPE patients was lower as the concentration of LDH increased.

Emerging evidence shows critical contribution of HER2 to poor prognosis and resistance to therapy in lung cancer (Hirsch et al., 2002a; 2002b; Zinner et al., 2004; Xia et al., 2012). Oncogene HER2 is a well-known poor prognostic marker and therapeutic target in breast cancer (Harris et al., 2001, Mojtahedi et al., 2011). The reported positivity for HER2 in lung tumors ranges from 5% to 75%, and non-small lung cancers are thought to more overexpress HER2 compared to small-cell lung cancers (Hirsch et al., 2002a; 2002b; Xia et al., 2012; Panagiotou et al., 2013). Clinical trials on lung cancer patients with advanced stages has shown therapeutic efficacy of anti-HER2 therapies only in patients with higher score of HER2 expression. Sequential screening consisting of serum HER2 ELISA followed by immunohistochemistry (IHC) analysis has been suggested for finding patients with higher score of HER2 expression (Zinner et al., 2004). To reduce the cost of IHC, it remains to identify other efficient and simpler screening systems.

Normal cells mostly depend on mitochondrial

*[1]Department of Surgery, Namazi Hospital, [2]Department of Internal Medicine, [3]Department of Pathology, Faghihi Hospital, [4]Cancer Proteomics and Biomarkers Lab, Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran *For correspondence: mojtahedizahra@hotmail.com*

oxidative phosphorylation, which consumes oxygen and glucose to produce energy. In contrast, cancer cells mostly employ glycolysis, the aerobic breakdown of glucose, for energy production (Warburg effect) (Zhao et al., 2009). Recent evidence demonstrates direct link of HER2 to glycolysis, and that HER2-overexpressing cells possessed a significantly higher level of glycolysis when compared to the HER2-low-expressing cells. It was further shown that overexpression of HER2 increased the expression of glycolysis-regulating molecules lactate LDH (Zhao et al., 2009; Bollig-Fischer et al., 2011).

The underlying mechanisms of increased LDH and decreased glucose levels in pleural space in poorer MPE need to be clarified. We hypothesized that HER2 expression might be an underlying reason for decreased glucose level and increased LDH levels in poorer MPE, and therefore these two biochemical tests could be screening tools for further finding HER2-positive patients in lung cancer using IHC. In the present study, LDH and glucose levels in newly diagnosed non-small cell lung cancer patients were measured and their associations with HER2 expression were determined.

Materials and Methods

Samples

The study was approved by the Ethics Committee of Shiraz University of Medical Sciences. Precipitants were informed that their blood samples would be used for research projects. Pleural effusions were sampled by thoracentesis or thoracoscopy from patients suspicious to malignancy before any treatments. Sera were drawn at the time of pleural effusion collection by venous puncture method. Pleural effusion and serum samples were centrifuged at 1000× g for 15 min at 4°C, and the supernatant was stored at -70°C until use. Bronchoscopy, thoracoscopy, or surgeries were sources for tissue specimens.

Collectively, 42 male non-small lung cancer patients with mean age 65.5±15.1, which had simultaneous pleural and serum samples and a tissue section, were enrolled in the study. Of them, 29 had adenocarcinoma and 13 had SCC, confirmed by a pathologist. Mean age of the adenocarcinoma and SCC patients were 66.3±14.8, and 64.6±14.8 yrs, respectively.

HER2 assay

The status of HER2 was checked by the semi-quantitative immunohistochemical HercepTest. Tumors were scored on a 0 to 3+ scale according to the criteria defined by the Ministry of Health of Iran. Sore 0: no staining or membrane staining in <10% of tumor cells; 1+: faint membrane staining in >10% of tumor cells; 2+: weak or moderate complete membrane staining in >10% of tumor cells; 3+: strong, complete membrane staining in >10% of tumor cells.

Lactate dehydrogenase and glucose assays

Lactate dehydrogenase and glucose photometric assay kits were purchased from Biorexfars (Iran) and Pars-azmoon (Iran). The adult normal value in serum for LDH was 161-322 U/L (at 37°C), and for glucose was 74-127 mg/dl.

Data analysis

The nonparametric tests, the Mann-Whitney U or the Kruskal Wallis test, were employed for statistical analyses. The data were analyzed using SPSS software (version 11.5.0; SPSS, Chicago, IL, USA). Findings were considered significant at a p value less than 0.05.

Results

In the present study, HER2 positivity was studied in Iranian non-small lung cancer patients. The HER2 expression was then compared to pleural and serum LDH and glucose levels. Of 42 patients, 29 (69.0%) had adenocarcinoma and 13 (31%) had SCC. Twenty-eight (66.7%) were HER2-negative. Fourteen (33.3%) were HER2- positive, of them, 9 (21.4%) samples weakly stained for HER2 (1+) and 5 (11.9%) moderately stained (2+). None of the patients had a strong positive result (3+). The difference of HER2 positivity between adenocarcinoma and SCC were not statistically significant (Table 1).

In 20 out of the 42 patients, simultaneous serum and

Table 1. Status of HER2 in Adenocarcinoma and Squamous Cell Carcinoma of the Lung

	Adenocarcinoma	Squamous cell carcinoma	p value
HER2-negative	20 (68.9%)	8 (61.5%)	0.86[a]
HER2-positive (1+)	6 (20.7%)	3 (23.1%)	
HER2-positive (2+)	3 (10.4%)	2 (15.4%)	
HER2-positive (1+ or 2+)	9 (31.1%)	5 (38.5%)	0.63[b]

[a]p value is given for the chi-square tests calculated on the 2×3 table (HER2-negative, HER2-positive (1+) and HER2-positive (2+) groups); [b]p value is given for the chi-square tests calculated on the 2×2 table, comparing the HER2-negative group with the HER2-positive (1+ or 2+) group

Table 2. Tumor Type, HER2 Status, Age and Mean Levels of LDH and Glucose in Serum (s) and Pleural Effusion (p) of the 20 Lung Cancer Patients

Tumor type[a]	HER2 stratus	Age (yr)	sLDH (U/L)	pLDH (U/L)	p/sLDH	sGlucose (mg/dl)	pGlucose (mg/dl)
A	0	81	540	410	0.75	90	42
A	1+	56	441	320	0.72	95	40
A	2+	48	495	410	0.82	85	38
A	0	63	486	250	0.51	80	62
A	1+	58	420	310	0.73	84	63
A	0	77	546	340	0.62	75	40
A	0	86	159	107	0.67	110	89
A	0	81	260	117	0.45	125	78
A	1+	40	410	350	0.85	76	35
A	0	79	267	230	0.86	84	50
S	0	56	325	275	0.84	91	40
A	1+	75	331	160	0.48	95	42
S	2+	90	485	390	0.8	73	31
A	0	53	250	143	0.57	65	34
S	0	83	546	440	0.8	93	75
A	0	83	435	153	0.35	95	78
A	0	52	295	154	0.52	125	90
A	1+	79	375	283	0.75	113	84
A	1+	72	524	430	0.82	84	41
A	2+	30	543	253	0.46	96	39

[a]Adenocarcinoma, A; S, Squamous cell carcinoma

Table 3. Mean and Median Levels of LDH and Glucose in Serum (s) and Pleural Effusion (p) in HER2-negative (HER2-), HER2-positive (HER2+) Lung Cancer Patients

		HER2- (n=13)	HER2+ (1+) (n=6)	HER2+ (2+) (n=3)	HER2+ (1+ or 2+) (n=9)	p value[a]	p value[b]	pvalue[c]
sLDH(U/L)	Mean±SD	373.5±140.5	416.8±65.1	507.6±31.0	447.1±70.4	0.28	0.3	0.29
	Median (range)	325 (159 –546)	415.0 (331-524)	495.0 (485 –543)	441.0 (331 –543)			
sGlucose(mg/dl)	Mean±SD	93.9±19.2	91.1 ±12.9	84.6±11.5	89.0±12.1	0.89	0.79	0.65
	Median (range)	91 (65-125)	89.5 (76-113)	85 (73-96)	85 (73-113)			
pLDH (U/L)	Mean±SD	238.0±117.2	308.8±88.6	351.0±85.4	322.8±84.7	0.17	0.06	0.17
	Median (range)	117.2 (107-440)	315 (160-430)	390 (253-410)	320 (160-430)			
pGlucose (mg/dl)	Mean±SD	61.6±21.1	50.8±18.9	36.0±4.3	45.8±16.8	0.05	0.08	0.02
	Median (range)	62 (34-90)	41.5 (35-84)	38 (31-39)	40 (31-84)			
p/sLDH	0.63	0.72	0.69	0.71	0.67	0.4	0.76	

[a]Kruskal Wallis test was used to calculate the differences among HER2-, HER2+ (1+), and HER2+ (2+) groups; [b]Mann-Whitney U test was used to calculate the differences between HER2- and HER2+ (1+or 2+) groups; [c]Mann-Whitney U test was used to calculate the differences between HER2- and HER2+ (2+) groups

pleural effusions samples were available. Table 3 indicates mean and median serum and pleural levels of LDH and glucose and the HercepTest results in these 20 patients. Pleural and serum LDH mean levels were increased with the scale of HER2 positivity, but did not reach statistical significance. Means of glucose levels were decreased with the scale of HER2 positivity only in pleural space. The difference in glucose levels among three groups revealed a p value almost significant (p=0.05). Comparison between HER2-negative and HER2-positive (1+ or 2+) groups revealed a p value near statistical significance (p=0.08), which became significant when comparison made between HER2-negative group and HER2-positive patients scored at 2+ (p=0.02). As indicated in Table 2, all patients in the latter group had glucose levels below 40 mg/dl.

Discussion

Evidence shows direct link of HER2 to increased glycolysis, a hallmark of cancer cell metabolism. HER2 has been demonstrated to activate signaling molecules that may regulate glucose metabolism, notably the critical glycolysis enzyme LDH (Zhao et al., 2009). HER2 overexpression, high LDH and low glucose pleural levels are all have been associated with poor prognosis in lung cancer in several studies (Sanchez-Armengol and Rodriguez-Panadero, 1993; Hirsch et al., 2002; Bielsa et al., 2008). Here, we investigated HER2 expression in lung cancer, and compared it to pleural and serum levels of LDH and glucose.

In our study, 33.3% of the patients were HER2-positive, of them, 21.4% were 1+, and 11.9% were 2+, and no patient was 3+. Data regarding status of HER2 in Iranian lung cancer patients is limited to the soluble form of HER2. Serologic analysis has detected increased levels of soluble HER2 in sera from 14% of SCC patients (Ghayumi et al., 2006). However, the soluble form may have low sensitivity for IHC. It has been shown in breast cancer, the best known cancer regarding HER2 expression, only 22% of patients with HER2 in the primary tumor shed HER2 into the serum (Harris et al., 2001). The frequency of HER2 overexpression in lung cancer tissues has been mostly studied in adenocarcinoma and SSC types in other populations and the reported frequencies of HER2 overexpression widely vary (Hirsch et al., 2002a; 2002b; Xia et al., 2012). For example, in Chinese lung cancer patients, Xia et al. (2012), reported the overexpression of HER2 in 74.3% of non-squamous cell carcinoma and 53.7% of squamous cell carcinoma without mentioning the score of HER2 staining (Xia et al., 2012). In the study conducted in the USA, Hirsch et al. (2002), found that 42% and 17.6% of adenocarnima and SCC cases were HER2 positive, respectively (8%, 1+; 27%, 2+; and 7%, 3+, in adenocarnima and 16.8%, 1+; 0.8%, 2+; and 0%, 3+ in SCC) (Hirsch et al., 2002). The discrepant results may be caused by differences in tissue processing, the employed antibodies, definition of positive results and study populations (Hirsch et al., 2002). It is worth mentioning that lung cancer most often have an 'intermediate' staining pattern (1+//2+/) and less often have 0 or 3 that is in contrast to staining results pertaining to breast cancer (Hirsch et al., 2002).

There is a general agreement on the association of low glucose and high LDH levels with poor prognosis in MPE, but the underlying mechanisms need to be completely understood (Sanchez-Armengol and Rodriguez-Panadero, 1993; Bielsa et al., 2008). It has been suggested that the elevated levels of LDH in MPE as a predictor of poor prognosis probably mirrors a higher degree of necrosis in the pleural cavity (Bielsa et al., 2008). Over utilization of glucose in pleural space rather than glucose transport defect have been shown to result in low glucose levels in MPE (Limthongkul, 1989). Here, in HER2-positive group compared to HER2-negative group, we found elevated pleural and serum LDH levels and decreased pleural glucose levels that reached a statistical significance in case of difference of pleural glucose levels between HER2-negative group and HER2-positive patients scored at 2+. This group all had glucose level under 40 mg/dl (Table 2).

Previously, it had been demonstrated that only those lung cancer patients with higher score of HER2 most respond to anti-HER2 therapy. Sequential screening consisting of serum HER2 ELISA followed by IHC analysis in only those lung cancer patients with increased serum HER2 levels had been suggested for reducing the cost of IHC (Zinner et al., 2004). Further research is warranted to investigate the possible employment of low pleural glucose level (less than 40 mg/dl) as a screening tool for finding patients with higher score of HER2expression using IHC.

We have demonstrated for the first time the association of HER2 with low glucose levels in MPE caused by lung

cancer. The limitation of our study was the low number of patients. It is worth mention that 65% of cancer patients presented by pleural effusion are diagnosed through cytology of pleural fluid which may not have further indication for diagnostic tissue biopsy or therapeutic surgery (Heffner and Klein, 2008). Therefore, most of lung cancer patients presented by MPE do not likely have any tissue specimen for determination of HER2. On the other hand, lung cancer in Iran is not as common as Western countries, although the survival is worse (Masoompour et al., 2011, Zahir and Mirtalebi, 2012). Re-conducting our study in countries with higher rates of lung cancer would overcome the limitation of our investigation.

In conclusion, our study shows the significant association of low glucose levels in pleural space with tissue expression of HER2 in lung cancer. If our study is confirmed in other populations and in larger sample size, the possible employment of low pleural glucose levels for finding HER2-positive cases in lung cancer is plausible.

Acknowledgements

This research was funded by grants from Shiraz University of Medical Sciences (88-01-31-1804) and Shiraz Institute for Cancer Research. No conflict of interest is declared by all authors.

References

Bielsa S, Salud A, Martinez M, et al (2008). Prognostic significance of pleural fluid data in patients with malignant effusion. *Eur J Intern Med*, **19**, 334-9.

Bollig-Fischer A, Dewey TG, Ethier SP (2011). Oncogene activation induces metabolic transformation resulting in insulin-independence in human breast cancer cells. *PLoS One*, **6**, 17959.

Ernam D, Atalay F, Hasanoglu HC, Kaplan O (2005). Role of biochemical tests in the diagnosis of exudative pleural effusions. *Clin Biochem*, **38**, 19-23.

Ghayumi SMA, Aghasadeghi K, Doroudchi M, Ghaderi A (2006). Determination of soluble HER-2/neu (sher-2/neu) in iranian patients with lung cancer. *Iran J Immunol*, **3**, 61-5.

Harris LN, Liotcheva V, Broadwater G, et al (2001). Comparison of methods of measuring HER-2 in metastatic breast cancer patients treated with high-dose chemotherapy. *J Clin Oncol*, **19**, 1698-706.

Heffner JE, Klein JS (2008). Recent advances in the diagnosis and management of malignant pleural effusions. *Mayo Clin Proc*, **83**, 235-50.

Hirsch FR, Franklin WA, Bunn PA (2002a). What is the role of HER-2/neu and trastuzumab (Herceptin) in lung cancer? *Lung Cancer*, **36**, 263-4.

Hirsch FR, Varella-Garcia M, Franklin WA, et al (2002b). Evaluation of HER-2/neu gene amplification and protein expression in non-small cell lung carcinomas. *Br J Cancer*, **86**, 1449-56.

Limthongkul S (1989). The pathogenesis of low pleural fluid glucose in acidotic malignant pleural effusions. *J Med Assoc Thai*, **72**, 492-7.

Masoompour SM, Yarmohammadi H, Rezaianzadeh A, Lankarani KB (2011). Cancer incidence in southern Iran, 1998-2002: results of population-based cancer registry. *Cancer Epidemiol*, **35**, 42-7.

Mojtahedi Z, Safaei A, Yousefi Z, Ghaderi A (2011). Immunoproteomics of HER2-positive and HER2-negative breast cancer patients with positive lymph nodes. *OMICS*, **15**, 409-18.

Panagiotou I, Georgiannos SN, Tsiambas E, et al (2012). Impact of HER2 and PTEN simultaneous deregulation in non-small cell lung carcinoma: correlation with biological behavior. *Asian Pac J Cancer Prev*, **13**, 6311-8.

Pirker R, Minar W (2010). Chemotherapy of advanced non-small cell lung cancer. *Front Radiat Ther Oncol*, **42**, 157-63.

Sanchez-Armengol A, Rodriguez-Panadero F (1993). Survival and talc pleurodesis in metastatic pleural carcinoma, revisited. Report of 125 cases. *Chest*, **104**, 1482-5.

Xia Q, Zhu Z, Wang J, et al (2012). Expression and association of HER2 with prognosis in early-stage (T1-T2N0M0) non-small cell lung cancer. *Tumour Biol*, **33**, 1719-25.

Zahir ST, Mirtalebi M (2012). Survival of patients with lung cancer, Yazd, Iran. *Asian Pac J Cancer Prev*, **13**, 4387-91.

Zhao YH, Zhou M, Liu H, et al (2009). Upregulation of lactate dehydrogenase A by ErbB2 through heat shock factor 1 promotes breast cancer cell glycolysis and growth. *Oncogene*, **28**, 3689-701.

Zinner RG, Glisson BS, Fossella FV, et al (2004). Trastuzumab in combination with cisplatin and gemcitabine in patients with Her2-overexpressing, untreated, advanced non-small cell lung cancer: report of a phase II trial and findings regarding optimal identification of patients with Her2-overexpressing disease. *Lung Cancer*, **44**, 99-110.