Association between Serum Epidermal Growth Factor Receptor and Cyclooxygenase-2 Levels in Patients with Non-small Cell Carcinoma of Lung

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Abstract

Relation of inflammation and cancer can be proven in most of the studies. Epidermal growth factor receptor (EGFR) overexpression is one of the commonest causes of non-small cell carcinoma of lung cancer (NSCLC). Cyclooxygenase-2 (COX-2) enzyme and its products; prostaglandin, prostacyclin, thromboxane are involved in inflammation. The aim of the study was to determine the association between serum EGFR and cyclooxygenase-2 levels in patients with NSCLC and healthy controls. It was a cross-sectional analytic study. This study included 53 patients diagnosed as NSCLC (3 adenocarcinoma and 50 squamous cell carcinoma (SCC) of lung patients) and 16 apparently controls. Serum EGFR and COX-2 levels were determined by ELISA.

Serum EGFR levels of healthy controls and NSCLC patients were 3.56±0.48 ng/ml and 170.10±13.80 ng/ml, respectively. In patients with NSCLC, serum EGFR of SCC and adenocarcinoma lung were 172.10±14.30 ng/ml and 137.40 ±64.70 ng/ml, respectively. Serum COX-2 levels of healthy controls and NSCLC patients were 0.62±0.15 ng/ml and 13.21±3.17 ng/ml. In patients with NSCLC, serum COX-2 levels of SCC and adenocarcinoma lung were 13.60±3.34 ng/ml and 6.69±5.52 ng/ml, respectively.

There is a significant association between serum EGFR and COX-2 levels in NSCLC patients ($X^2 = 7.854$, $p = 0.005$). Mean level of serum EGFR (170.10 ng/ml) and COX-2 (13.21 ng/ml) are set as cut off values for categorization of low and high groups. There was no correlation between serum EGFR and COX-2 in healthy controls ($r = -0.036$, $p = 0.894$) and NSCLC patients ($r = 0.161$, $p = 0.250$). After excluding 4 outliers, the correlation between serum EGFR and COX-2 levels in NSCLC patients became significant ($r = 0.454$, $p = 0.001$). Correlation between serum EGFR and COX-2 levels in SCC patients was not significant statistically ($r = 0.142$, $p = 0.325$) but after excluding the 4 outliers, it became significant statistically ($r = 0.418$, $p = 0.004$). There was positive correlation in adenocarcinoma ($r = 0.999$, $p = 0.021$). These findings indicated that EGFR and COX-2 play an important role in carcinogenesis of lung and are positively associated.

INTRODUCTION

Tumorigenesis is a multi-step process of transformation from normal bronchial epithelium to overt lung cancer. The various molecular changes in this process result in dysregulation of key pathways involved in cellular growth and apoptosis. Major classes of human cancer genes targeted by genetic lesions are the protooncogenes and tumor suppressor genes[1].

Epidermal growth factor receptor (EGFR) is one of the products of protooncogenes and is a kind of receptor tyrosine kinase expressed on the surface of epithelial cells. EGFR regulates vital cellular processes such as proliferation, migration, survival and angiogenesis[2].

Many chronic skin inflammation or irritations are known to be associated with increased prevalence of squamous cell carcinoma[3]. Cyclooxygenase-2 (COX-2) is an inducible enzyme stimulated by cytokines, growth factors, oncogenes, or tumor promoters during inflammation or

malignancy. COX-2 over-expression is increased in association with decreased apoptosis, increased tumor invasiveness, immunosuppression and angiogenesis[4].

Epidermal growth factor receptor and cyclooxygenase-2 share common signaling pathway[5] in carcinogenesis in vitro studies. But the association between EGFR and COX-2 is still controversial and the previous studies were done in tissue by immunohistochemistry method.

MATERIAL AND METHDS

It was cross-sectional descriptive study conducted from December 2010 to June 2014. A total 53 numbers of patients diagnosed as NSCLC by histology and cytology reports by pathologist were collected from Department of Respiratory Medicine and Department of Thoracic Surgery, Yangon General Hospital were included. Sixteen numbers of apparently healthy adults who lived in Lanmadaw Township and worked at Department of Biochemistry, University of Medicine 1 (Yangon) were selected as control. Both men and women patients and normal subjects were included. But the patients who have renal or hepatic failure with high serum creatinine or liver enzymes, pulmonary tuberculosis, autoimmune disease, other inflammatory diseases and other malignancies and patients who were taking NSAID and or steroid drugs within 3 weeks were excluded from the study.

After taking informed consent, history taking and physical examination, 5 ml of blood were collected and serum were stored at -80°C before analysis. Determination of serum EGFR and COX-2 were done at Nuclear Medicine Research Division, Department of Medical Research by ELISA method which was a quantitative sandwich immunoassay and both kits were products of Uscn Life Science, USA.

Data entry was done in Microsoft Excel and data analysis was done by using the Statistical Package for Social Science (SPSS) software version 16. Standard statistical methods were applied for the calculation of mean, standard error of the mean and standard deviation. The numbers of patients with high or low serum EGFR or COX-2 were calculated by using mean serum level of EGFR or COX-2 as cut off values. Then the association between serum EGFR and COX-2 levels was analyzed by Chi square test. Correlation of serum EGFR and COX-2 levels was tested using Pearson correlation coefficient (r). Significant level was decided if the probability levels of all tests were <0.05.

Ethical consideration

The proposal of this study was approved by Ethic and Research Committee, University of Medicine 1 on 18th January, 2012.

RESULTS

Age (mean ± SD) of healthy controls, patients with NSCLC, SCC and adenocarcinoma of lung were 56.38±10.17 years (range= 40-73), 60.83±9.62 years (range = 27-79), 60.66±9.85 years (range = 27-79) and 63.67±3.51 years (range = 60-67), respectively. There is no significant difference in age between healthy controls and patients with NSCLC or SCC or adenocarcinoma of lung ($p = 0.134$, $p = 0.152$ and $p = 0.052$) respectively. In healthy control group, majority (63%) were females but in NSCLC patients, majority (70%) were males. In calculating association, mean level of serum EGFR (170.10 ng/ml) and COX-2 (13.21 ng/ml) were set as cut off values for categorization of low and high groups.

Table 1. Serum EGFR and COX -2 levels in patients with NSCLC and healthy controls

Parameter	Mean ± SEM (Range)
Serum EGFR level (ng/ml)	Healthy control (n=16) 3.56 ± 0.48 (0.19 - 5.47)
	NSCLC patients (n = 53) 170.10 ± 13.80 (4.14 - 439.20)
	SCC patients (n=50) 172.10 ± 14.30 (4.14 – 439.20)
	Adenocarcinoma patients (n = 3) 137.40 ± 64.70 (64.70 - 266.40)
Serum COX-2 level (ng/ml)	Healthy control (n=16) 0.62 ± 0.15 (0.00 - 2.03)
	NSCLC patients (n = 53) 13.21 ± 3.17 (0.49 - 145.70)
	SCC patients (n=50) 13.60 ± 3.34 (0.49 - 145.70)
	Adenocarcinoma patients (n = 3) 6.69 ± 5.52 (0.78 - 17.73)

Table 2. Association between serum EGFR and COX-2 levels in NSCLC patients

EGFR level	No. of subjects		Total number	X^2 value	p value
	Low COX-2 level	High COX-2 level			
Low EGFR level	22	4	26	7.854	0.005
High EGFR level	13	14	27		
Total number	35	18	53		

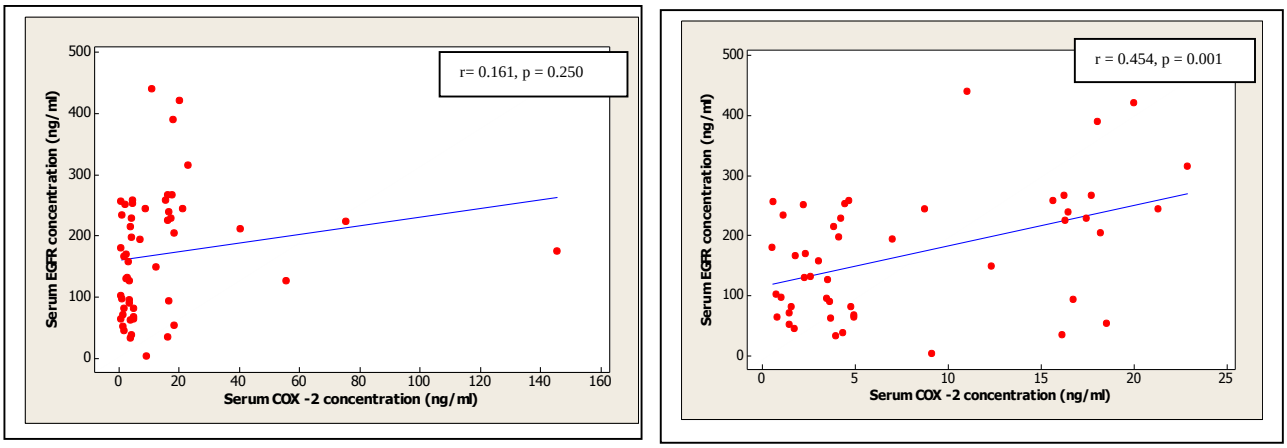

Figure 1a & b. Correlation between serum EGFR and COX-2 levels in NSCLC patients before and after excluding 4 outliers

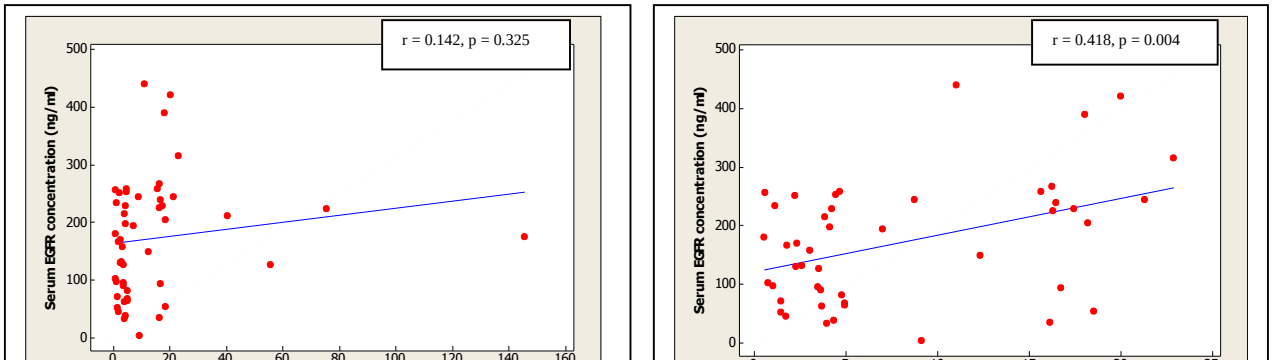

Figure 2a & b. Correlation between serum EGFR and COX-2 levels in SCC patients before and after excluding 4 outliers

DISCUSSION

Lung cancer was the most commonly diagnosed cancer world wide and had 13.0% (1.8 million) of the total new cancer cases. It was also the most common cause of cancer death and had 19.4% (1.6 million) of the total cancer-related deaths [6].

EGFR normally involves in cell growth, proliferation, cell division, differentiation and apoptosis. Over-expression or mutation of EGFR activates cellular signaling pathways that induce characteristics of cancer cells, including angiogenesis, metastasis, and invasiveness. COX-2 and its products prostaglandin are known to play an important role in carcinogenesis by stimulating growth, survival, invasion, metastasis, and angiogenesis of tumor cells.

Although the actions of COX-2 and EGFR are closely related in cells, expression patterns of COX-2 and EGFR are highly variable among cancer cell types and frequently seem to be independent of each other. Thus, determining the precise relationship between the two proteins in a specific cancer type could provide useful clues for the development of new drugs or approaches e.g., combined targeted strategies[7].

In the present study, there was a significant association between serum EGFR and COX-2 levels in NSCLC patients. There was also a significant positive correlation between them in NSCLC and SCC patients after excluding 4 outliers although it was not significant before. These 4 patients were SCC lung cases and their COX-2 levels were very much higher than the mean level of SCC patients although their EGFR levels were a little higher than the mean level. One patient had congestive liver and bilateral nephropathy in abdominal ultrasound report and another patient had parotid tumor. There were no significant findings in other 2 patients apart from smoking history of 33 and 45 years duration. It may be due to other inflammatory disorders apart from NSCLC. Although positive correlation was found in adenocarcinoma lung, it can't be counted due to small sample size. There was no correlation between serum EGFR and COX-2 levels in normal subjects.

The result of the present study was consistent with other studies[8, 9, 10]. In these studies, EGFR expression was positively correlated with COX-2 expression although EGFR and COX-2 expression were detected by IHC. The correlation might be due to involvement of cross-talk between EGFR and COX-2 signaling. Transactivation of EGFR represents the paradigm for cross-talk. The mediators involved in EGFR transactivation depend on the activated G-protein coupled receptor (GPCR), the cell type, and the specific physiological state of the cell[11].

COX-2 transactivates EGFR through production of prostaglandins mainly PGE_2. Via EP4 receptor, PGE_2 increases the expression of EGFR ligand; amphiregulin through cAMP-response element binding proein.[12] PGE_2 also causes release of membrane bound EGFR ligands by activating matrix metalloprotease (MMP). In addition, PGE_2 activates non-receptor tyrosine kinase Src by increasing intracellular calcium. Src activates intracellular EGFR by phosphorylation. [13]

EGFR activation results COX-2 gene expression via transcription factor AP1. Activated EGFR also increases the level of PGE_2 by inhibiting the expression of 15-hydroxyprostaglandin dehydrogenase which is responsible for catabolism of PGE_2.[14]

Activated EGFR activates urokinase type plasminogen activator leading to activation of plasmin which activates MMP. MMP can increase the release of membrane bound EGFR ligands resulting in more activation of EGFR.[15] EGFR activation by ligand dependent or ligand independent can activate transcription of EGFR ligand genes through Ras-MAPK pathway[13]. EGFR ligands (i.e., EGF and TGF-α) itself can enhance release of membrane bound EGFR extracellular domain[16] and leads to increased serum EGFR level.

But, one study[5] discovered that there was no correlation between EGFR and COX-2 expression in NSCLC. EGFR can be activated by direct ligand binding or other stimuli. EGFR can be activated by other receptor tyrosine kinase agonists, cytokines, chemokines and cell adhesion elements, UV and gamma radiation, osmotic shock, membrane depolarization, heavy metal ions and radical-generating agents such as hydrogenperoxide[5].

The present study showed that there was an association between serum EGFR and COX-2 in SCC patients and they were positively correlated. It indicated that both EGFR and COX-2 have involved in development of NSCLC and relation of inflammation and carcinogenesis.

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