Myanmar Health Sciences Research Journal, Vol. 28, No. 1, 2016

Comparison of Serum Epidermal Growth Factor Receptor and Cyclooxygenase-2 Levels in Patients with Non-small Cell Carcinoma of Lung and Normal Subjects

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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 compare the serum epidermal growth factor receptor and cyclooxygenase-2 levels between patients with non-small cell carcinoma of lung and healthy controls. This study included 53 patients diagnosed as NSCLC and 16 apparently healthy controls. In 53 patients, 3 patients were diagnosed as adenocarcinoma and 50 patients were diagnosed as squamous cell carcinoma (SCC) of lung. In both subjects, serum EGFR and COX-2 levels were determined by ELISA. The mean serum EGFR and COX-2 levels of NSCLC patients were significantly higher than those of healthy controls (170.10±13.80 *vs.* 3.56±0.48 ng/ml) and (13.21±3.17 *vs.* 0.62± 0.15 ng/ml), respectively ($p<0.001$ in both). Both the mean serum EGFR and COX-2 levels of SCC patients (172.10±14.30 ng/ml and 13.60±3.34 ng/ml) were significantly higher than those of healthy controls ($p<0.001$ in both). Both serum EGFR levels and COX-2 levels of patients with adenocarcinoma of lung (137.40±64.70 ng/ml and 6.69±5.52 ng/ml) were not significantly different with those of healthy controls ($p=0.174$ and $p=0.386$), respectively. The mean serum EGFR and COX-2 levels of SCC patients were higher than those of adenocarcinoma patients but they were not significant ($p=0.653$ and $p=0.363$), respectively. These findings indicated that EGFR and COX-2 play an important role in carcinogenesis of lung.

Key words: Epidermal growth factor receptor, Cyclooxygenase-2, Non-small cell carcinoma of lung, Prostaglandin

INTRODUCTION

Carcinoma of the lung is the leading cause of death worldwide and has a poor prognosis. Dysregulation of key pathways involved in cellular growth and apoptosis results in tumorigenesis. Epidermal growth factor receptor (EGFR) is one of the products of protooncogenes. EGFR 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.[1]

Many chronic skin inflammation or irritations are known to be associated with increased prevalence of squamous cell carcinoma.[2] Cyclooxygenase-2 (COX-2) is an inducible enzyme stimulated by cytokines, growth factors, oncogenes, or tumor promoters during inflammation or malignancy. The carcinogenic effect of COX-2 mainly exerted through the increase level of prostaglandin.[3] It results in decreased apoptosis, increased tumor invasiveness, immunosuppression and angiogenesis.[4]

Involvement of these two molecules in carcinogenesis is determined by immunohistochemistry (IHC) method *in vitro* studies

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or cancers other than lung cancer. In this study, determination of EGFR and COX-2 levels were done by ELISA methods in the serum of patients with NSCLC.

MATERIALS AND METHODS

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 the 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 had 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 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 (Lower Myanmar) 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. Comparison of the serum EGFR and COX-2 levels was analyzed by unpaired 't' test. Significant level was decided if the probability levels of all tests were <0.05.

Ethical consideration

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

RESULTS

Table1. Comparison of serum EGFR and COX-2 levels of healthy control and NSCLC patients

Parameter		p value
Serum EGFR level (ng/ml)		
Healthy control (n=16) 3.56±0.48 (0.19-5.47)	NSCLC# (n=53) 170.10±13.80 (4.14-439.20)	<0.001
Healthy control (n=16)	SCC# (n=50) 172.10±14.30 (4.14-439.20)	<0.001
Healthy control (n=16)	Adenocarcinoma# (n=3) 137.40±64.70 (64.70-266.40)	0.174
SCC# (n=50)	Adenocarcinoma# (n=3)	0.653
Serum COX-2 level (ng/ml)		
Healthy control (n=16) 0.62±0.15 (0.00-2.03)	NSCLC# (n=53) 13.21±3.17 (0.49-145.70)	<0.001
Healthy control (n=16)	SCC# (n=50) 13.60±3.34 (0.49-145.70)	<0.001
Healthy control (n=16)	Adenocarcinoma# (n=3) 6.69±5.52 (0.78-17.73)	0.386
SCC# (n=50)	Adenocarcinoma# (n=3)	0.363

#=Patients, data were shown in Mean±SEM (range).

Age (mean±SD) of healthy controls, patients with NSCLC, SCC and adenocarcinoma of lung are 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.

DISCUSSION

Lung cancer was the most commonly diagnosed cancer worldwide and had 12.9% (1.8 million) of the total new cancer cases. It was also the most common causes of cancer death and had 19.4% (1.6 million) of the total cancer-related deaths.[5]

For therapeutic purposes, carcinomas of the lung are classified into two broad groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC and NSCLC are clinically and genetically distinct. SCLCs are best treated by chemo-therapy because all are metastatic to other tissues. By contrast, NSCLCs are curable by surgery if limited to the lung.

EGFR normally involves in cell growth, proliferation, cell division, differentiation and apoptosis. Over-expression or mutation of EGFR activates cellular signaling path-ways that induce characteristics of cancer cells, including angiogenesis, metastasis, and invasiveness. Thus, EGFR is as target of therapy and have been implicated in disease progression, decreased survival, and resistance to cytotoxic agents or radiation. COX-2 and its products prostaglandin are known to play an important role in carcinogenesis by stimulating growth, survival, invasion, metastasis, and angio-genesis of tumor cells.

In the present study, the mean serum EGFR level in healthy control was 3.56± 0.48 ng/ml and in NSCLC patients was 170.10±13.8 ng/ml. The mean serum EGFR level of NSCLC patients was significantly higher than that of healthy controls ($p<0.001$). The mean serum EGFR level in SCC lung patients was also significantly higher than that of healthy controls ($p<0.001$). The mean serum EGFR level of adenocarcinoma patients was much higher than that of healthy controls and it could not be concluded that it was not statistically significant ($p>0.174$) because the number of adenocarcinoma patients was only 3.

The similar results were seen in other studies, however, the method of EGR determination was different. Zhu, *et al* and Li, *et al* in China reported that EGFR expression in primary lung cancer tissues was significantly higher than those of the control although EGFR expression was detected by IHC method.[6, 7] In the study of Fujino, the mean EGFR level in primary non-small cell lung cancer tissues was significantly higher than in normal tissues but serum EGFR level was measured by using a competitive radiolig and binding assay.[8]

Increased serum EGFR level in carcinoma of lung may be due to EGFR over-expression. Over-expression of EGFR can occur by amplification or mutation of EGFR gene. But Dacic, *et al* found that EGFR over-expression in NSCLC was uncoupled with gene amplification.[9] Three polymorphisms that are associated with EGFR over-expression are a polymorphic dinucleotide repeat (CA simple sequence repeat 1 [CA-SSR1]) in intron one (lower number of repeats) and two single nucleo-tide polymorphisms in the promoter region, -216 (G/T or T/T) and -191 (C/A or A/A).[10]

There were also other studies which results were inconsistent with those of the present study. In the study of Lemos-Gonzalez and colleagues, the mean serum EGFR level of NSCLC patients was significantly decreased than that of healthy subjects.[11] Schneider, *et al* found that there was no significant difference in serum EGFR levels in patients with lung caners and healthy controls.[12] Decreased or no difference in serum EGFR level in carcinoma of lung may be due to different activity of metalloprotease enzymes. EGFR extracellular domains and its ligands are shed from membrane by membrane anchored proteases, a family of a disintegrin and metalloproteinase (ADAM). The proteolytic activity of ADAM can be influenced by factors such as osmotic and mechanical stress, G protein-coupled receptor activation, activation of proteinkinase C, increase in intracellular calcium, serum factors and growth factors.[13]

In the present study, serum EGFR levels in patients with SCC of the lung were higher than those of patients with adenocarcinoma lung but it can't be compared because the number of adenocarcinoma lung patients was only 3. The similar pattern of result was obtained, however, EGFR expression was determined on tumor cells by IHC method rather than ELISA. In the study of Veale,

et al in which EGFR was most commonly found in SCC (70%) followed by adenocarcinoma (50%).[14] Dacic and coworkers also found that EGFR protein expression was more common in SCC (26.2%) than in adenocarcinoma (11.1%) (p=0.0076).[9] EGFR expression in SCC of lung was more frequently associated with EGFR amplification than adenocarcinoma lung (14.5%) *vs.* (3.6%) cases (p=0.0208).

In the present study, the mean serum COX-2 level of NSCLC patients (0.62±0.15 ng/ml) was significantly higher than that of healthy controls (13.21±3.17 ng/ml) (p<0.001). In most of the studies, COX-2 expression was detected by IHC in lung tissues rather than serum by ELISA. Li and colleagues (2011) found that COX-2 expression was 90% for NSCLC tumors and was significantly higher than that for normal lung (0%).[7] Zhu, Liu and Wang detected that COX-2 expression in primary lung cancer tissues (52.8%) was significantly higher than those of the control (p<0.05).[6] In the present study, mean serum COX-2 level in patients with SCC of the lung was higher than that of patients with adenocarcinoma of the lung but it was not significant (p=0.363). It was not consistent with the studies that COX-2 expression was found more in adenocarcinoma than SCC of lung.[7, 15]

The higher serum COX-2 level in SCC than adenocarcinoma of lung in the present study may be due to large difference in number of cases between adenocarcinoma cases (n=3) and SCC cases (n=50). Another explanation for this discrepancy was COX-2 expression was measured by IHC in the previous studies and serum COX-2 was measured by ELISA in the present study. Since COX-2 enzyme is located at the luminal side of the endoplasmic reticulum and nuclear membrane, it can't be detected in the serum until the cells are destroyed.[16] Therefore, serum COX-2 level may not reflect its actual expression in cancer cells.

The findings of the present study indicated that both EGFR and COX-2 have implication in development of NSCLC and can be applied in further research in relation between cancer and inflammation. But in the present study, EGFR and COX-2 expressions couldn't be measured in the tissue samples. Therefore, applicability of measuring serum COX-2 as an additional serum marker for diagnosis of non small cell carcinoma of lungs is not sure although measuring serum EGFR may applicable. Further studies are necessary for confirmation.

ACKNOWLEDGEMENT

We gratefully thank to Professor Win Naing, Professor and Head, Department of Respiratory Medicine, YGH and Professor Maung Maung Khin, Professor and Head, Department of Thoracic Surgery, YGH for allowing us to study the patients under their care. We would like to express our sincere thanks to Dr. Aye Aye Yee, Head/ Research Scientist, Nuclear Research Division, Department of Medical Research (Lower Myanmar) and staff for their support.

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