A Study of the Relationship between the Blood Levels of the 8-Oxoguanine DNA Glycosylase, Smoking and Risk of Lung Cancer

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Abstract

Introduction: The role of The 8-oxoguanine DNA glycosylase (OGG1) is to delete DNA parts that have been damaged by oxygen radicals, thus preventing lung cancer.

Objective: This work is aiming to study the relationship between the variation in the blood levels of the 8-oxoguanine DNA glycosylase (OGG1), smoking and risk of lung cancer.

Material and Methods: This study included 36 patients diagnosed as bronchogenic carcinoma, divided pathologically into 30 non small cell lung cancer (NSCLC) patients and 6 small cell lung cancer (SCLC) patients, 40 non-smoker and 40 smokers’ healthy subjects, taken as control. They were subjected to medical history taking, clinical examination, chest radiography and Quantitative RT-PCR of mRNA levels of OGG1 in blood.

Results: There was a non significant increase in the mean level of the OGG1 in the non smoker group compared to the cancer and smokers’ groups (p>0.5). There was a non statistical significant decrease in the mean value of the OGG1 in the NSCLC compared to that of SCLC (p>0.5). There was statistically highly significant decrease in the mean value of the OGG1 in cases of adenocarcinoma compared to that in cases of squamous cell carcinoma and SCLC (p<0.0001).

Conclusion: The decreased in the levels of OGG1 in blood could be a risk factor for lung cancer and was associated with adenocarcinoma. A substantial fraction of lung cancer cases might result from a combination of smoking and reduced OGG1 level.

Key Words: OGG1 – RT PCT – Lung cancer.

Introduction

Among various histologic types of lung cancer, cigarette smoking had the strongest effects on small cell carcinoma, followed by squamous cell carcinoma and adenocarcinoma. There were only weak associations with the homozygous variant for all cell types of lung cancer combined and for adenocarcinoma of the lung [1]. Squamous and small cell carcinomas of the lung, which are related to smoking, may be regarded as diseases that are distinct from lung adenocarcinoma, which is less related to smoking. They have strikingly different molecular profiles, as reported in a previous study [2]. Although cigarette smoking is the major cause of lung cancer, only a small fraction of smokers (10%) ever develop lung cancer. This may be due to the variable quantity of cumulative smoke exposure among ever smokers. It may also be attributable to some genetic factor (s) which may reduce the effect of cigarette smoking on the risk of lung cancer [3].

The interactive effects of DNA repair genes and cigarette smoking history on lung cancer risk are worth additional investigation in future studies, as cigarette smoke contains numerous compounds that generate reactive oxygen species that can damage DNA directly or indirectly [4] via inflammatory processes [5,6]. Oxidants, either present in cigarette smoke and/or formed in the lungs of smokers, may trigger oxidative damage to DNA and cellular components, contributing to carcinogenesis. Free radical attack on DNA generates a multiplicity of DNA damage, including modified bases. Some of these modifications have considerable potential to damage the integrity of the genome. Although the quantitative relationship between the measured DNA damage and the development of cancer is lacking, evidence suggests that oxidants act at several stages in the malignant transformation of cells [7].

Lesions that persist in DNA cause mutations. The accumulation of mutations in critical genes,
namely, oncogenes, and tumor suppressor genes leads to the formation of cancer. It was therefore reasonable to assume that the efficiency of DNA repair will be an important factor in cancer risk. Efficient DNA repair mechanisms will keep mutations rate low, and reduce the risk of cancer, whereas weak DNA repair will lead to a faster increase of mutations, and to a higher cancer risk [8].

The 8-oxoguanine DNA glycosylase (OGG1) is one of the key DNA repair enzymes involved in the Base excision repair (BER) pathway in humans [9]. It recognizes the 8-oxoguanine modifications from both nuclear and mitochondrial DNA [10]. The role of OGG1 is to delete DNA parts that have been damaged by oxygen radicals, thus preventing lung cancer [11].

The excision of 8-oxoguanine residues by OGG1 protects against aberrant adenine-cytosine and guanine-thymine conversions that can lead to heritable mutagenesis, particularly in non-proliferative cells in which lesions accumulate by cell division [12].

Reduced OGG1 activity could be expected to be a risk factor in other smoking-related cancers. However, given the abundance of 8-oxoguanine and the suspected role of oxidative stress in cancer, reduced OGG1 activity might be associated with the risk of some other cancers as well [13]. But, there is a discrepancy about the distribution of the OGG1 polymorphism that might differ in the different ethnicities and environmental exposures that may have modified this associations [14-17].

Aim of the work:

The study aimed to detect the relationship between the variation in the blood levels of the 8-oxoguanine DNA glycosylase (OGG1), smoking and risk of lung cancer in Egypt.

Subjects and Methods

This study was collaboration between Cancer Institute Cairo University and National Research Center. A case-control study was conducted. It included 116 Egyptian males: 36 patients diagnosed pathologically as primary lung cancer and 80 apparently healthy subjects; 40 non-smoker and 40 smokers, included as controls. All subjects gave written informed consent. The study was approved by the ethical committee of the National Research Center. Exclusion criteria were patients with pulmonary fibrosis, acute interstitial pneumonia and receiving any anti-cancer treatment before enrollment. Exclusion criteria were previous history of cancer for control subjects, and previous smoking for non smokers controls.

All subjects included in the study were interviewed to fulfill personal and medical questionnaire, thorough clinical examination and chest radiography, and blood sampling for OGG1 mRNA quantification.

Quantitative RT-PCR of mRNA levels of the DNA repair gene, 8-oxo-guanine-DNA glycosidase (OGG1) in blood:

Total RNA was purified from 1.5ml EDTA stabilized full blood <4 h after sampling. Qiagen blood RNA isolation kit was used as recommended by the manufacturer yielding ~ 5µg total RNA.

Before running the TaqMan Gene Expression Assays, the isolated total RNA is used as a template for synthesis of single-stranded cDNA. For optimal performance, Applied Biosystems recommends using an Ambion® RNA isolation kit. Denmark.

Quantitative PCR was performed on: Step One- Applied Biosystems instrument in universal mastermix (Applied Biosystems). OGG1 primers were: OGG1 714F, 5'-aaa ttc caa ggt gtg cga ctg-3'; OGG1 796R, 5 '- ggt gtt gtt tgg ctg caa gga ggg cgg a a -3 '; probe, 5'- FAM- caa gac ccc atc gaa tgc ctt ttc tct tt- TAMRA-3' [18] obtained from Applied Biosystems.

PCR amplification was done using 5µl cDNA, 900 nM of each primer, 250 nM of the probe and 12.5 µl PCR Master Mix (2x Atlas HotTaq PCR Mix, BioAtlas, Gentaur Biotech., Germany).

Fig. (1): Gene Expression Plot (RQ vs Target).
Statistical analysis:

Data were analyzed using statistical package system SPSS version 14.0. The quantitative results were expressed as means ± standard deviation (SD). For comparing quantitative results between two groups, Independent t-test was used, and for more than two groups, ANOVA and post hoc least significant test (LSD) were used. Statistical analysis considered to be significant when \( p \)-value was 0.05.

Results

The included patients with primary lung cancer patients were diagnosed to be 30 cases diagnosed as non small cell lung cancer (NSCLC), (12 squamous cell carcinoma and 18 adenocarcinoma) and 6 cases diagnosed as small cell lung cancer (SCLC). The apparently control subjects (smokers and non-smokers) were matched for age with the lung cancer patients (Table 1).

Table (1): Statistical analysis of age distribution (years) in the 3 studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No</th>
<th>Age Mean</th>
<th>SD</th>
<th>( p )-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>40</td>
<td>50.4</td>
<td>7.3</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Smokers</td>
<td>40</td>
<td>54.3</td>
<td>8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung cancers</td>
<td>36</td>
<td>52.2</td>
<td>8.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean value of the OGG1 level in the lung cancer cases, the controls; non smokers and smokers, were 3.4±3.07 U, 7.0±2.40 and 4.5±5.97 U respectively. There was an increase in the mean level of the OGG1 in the non smoker group compared to the other two groups, without significant difference. There was also no statistical significant difference between the mean OGG1 mean value between smokers and lung cancer patients (Fig. 2).

There was a non statistical significant decrease in the mean value of the OGG1 in the SCLC patients compared to that of NSCLC (Table 2).

Table (2): Statistical analysis of the mean value of OGG1 between the SCLC and NSLC in the lung cancer group.

<table>
<thead>
<tr>
<th>OGG1</th>
<th>No</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCLC</td>
<td>6</td>
<td>4.17</td>
<td>2.48</td>
</tr>
<tr>
<td>NSCLC</td>
<td>30</td>
<td>3.30</td>
<td>0.579</td>
</tr>
</tbody>
</table>

There was a highly statistical significant decrease in the mean value of the OGG1 in cases of adenocarcinoma compared to that in cases of squamous cell carcinoma and SCLC (Table 3).

Table (3): Statistical analysis of the mean value of OGG1 between the different histological types of lung cancer.

<table>
<thead>
<tr>
<th>OGG1</th>
<th>No</th>
<th>Mean</th>
<th>SD</th>
<th>( F ) ratio</th>
<th>( p ) value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous</td>
<td>12</td>
<td>6.69</td>
<td>0.53</td>
<td></td>
<td></td>
<td>(Adeno)</td>
</tr>
<tr>
<td>Adeno- carcinoma</td>
<td>18</td>
<td>1.03</td>
<td>0.28</td>
<td>54.33 &lt;0.0001</td>
<td></td>
<td>(SCLC, Sq)</td>
</tr>
<tr>
<td>SCLC</td>
<td>6</td>
<td>4.17</td>
<td>2.48</td>
<td></td>
<td></td>
<td>(Adeno)</td>
</tr>
</tbody>
</table>

Discussion

Human 8-oxoguanine DNA N-glycosylase 1 (OGG1) plays an important role in repairing oxidative DNA damage induced by tobacco carcinogens [1]. OGG1 is a critical component of the BER pathway required for the removal of 8-Hydroxy-2'-deoxyguanosine (8-OHdG) lesion from DNA exposed to reactive oxygen species (ROS). The impaired repair activity of OGG1 protein was suggested to be a factor contributing to the high somatic mutation rate in human cells, since the accumulation of 8-OHdG cause mutations by mispairing with adenine during replication [9]. It has also been reported that lower enzymatic levels of OGG1 in peripheral lymphocytes correlated with an increased risk of lung cancer among smokers [19].

In the present work, we studied the blood level of the OGG1 gene among lung cancer cases, controls smokers and non smokers. The included subjects in three groups were Egyptian males and were matched in age. The results demonstrated an increase in the levels of the OGG1 in the non-smoker group compared to that of smokers and lung cancer groups. But, there was no statistical significant difference in the mean value of OGG1 between the three groups, most probably due to the large
variations in the values of OGG1 that results in high standard deviation (Fig. 2). This relation between the low level of OGG1 and lung cancer is in concordance with other studies who reported that lower enzymatic levels of OGG1 in peripheral lymphocytes correlated with an increased risk of lung cancer among smokers [1,19]. Moreover, it was found that smokers with low OGG1 activity were five to 10 times more likely to suffer from cancer lung than smokers who have normal OGG1 activity. This risk increased to 120 times when comparing the group to non-smokers with normal levels of the enzyme [11]. On the other hand, El-Zein et al., [13], demonstrated that baseline DNA damage and reduced OGG1 activity is similar among lung cancer cases and current smoker controls.

Our data demonstrated a very highly significant decrease in OGG1 level is associated with adenocarcinoma (1.03±0.28 U) compared to squamous cell carcinoma (6.69±0.53 U) and SCLC (4.17±2.48U) (Table 3).

Several studies agreed the association between the reduced OGG1 levels and activity and the occurrence of adenocarcinoma [20-22]. In contrast, Ito et al., [17] found a limited association between OGG1 polymorphism and adenocarcinoma and recommended an additional validation in larger and well designed studies.

On the other hand, several studies demonstrated this reduction in the OGG1 level and/ or activity in NSCLC in general. Paz-Elizur et al., [19] showed that reduced OGG1 activity is associated with the occurrence of NSCLC in an Israeli population. Results of a study in a Hawaiian population that comprised Caucasian and Japanese individuals found that the OGG1 Cys/Cys genotype was associated with both adenocarcinoma and squamous cell carcinoma risks, with the association being stronger for the latter [15]. In a Japanese population, an association between squamous cell lung cancer and the OGG1 Cys/Cys genotype was proved [14].

The findings of another study provided empirical evidence for synergistic effects of human OGG1 genotype and cigarette smoking on lung cancer risk, and the interactions were observed for all histological types of lung cancer. The interactions of human OGG1 genotype with heavy smoking were observed in both smoking-related (squamous and SCLC) and less-smoking-related (adenocarcinoma) lung cancers. Although the biological mechanism is not clear, it is possible that the human OGG1-involved base excision repair pathway may be one of the common pathogenic mechanisms for major types of lung cancer [1].

This discrepancy might reflect the fact that the studied population included subjects of different ethnicities and environmental exposures that may have modified the associations, especially given that the distribution of the OGG1 polymorphism differs among Chinese/Japanese and Caucasian populations [14-17].

Small cell carcinoma had wide confidence intervals because of the small number of subjects in this study. Future studies with a large sample of patients are needed to clarify the interactions between OGG1 level in blood and cigarette smoking in small cell carcinoma.

In conclusion: In Egypt, the decreased in the levels of OGG1 in blood could be a significant risk factor for lung cancer and was associated with adenocarcinoma. A substantial fraction of lung cancer cases might result from a combination of smoking and reduced OGG1 level.

Screening for smokers with low OGG1 level, followed by smoking cessation in these individuals, may lead to a decrease in the incidence of lung cancer. Further studies were also recommened in the different Egyptian populations.

References


