Association of IL-6 rs1800795 and IL-1β rs16944 Polymorphisms with non-small Cell Lung Cancer in the Egyptian Population: a Pilot Study

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Abstract

Lung cancer is a serious health and life issue, with the highest rates of incidence and mortality in the world. It is now clear that inflammation is a key factor involved in all aspects of carcinogenesis, notably lung cancer development. Genetic changes, including polymorphisms in inflammatory genes, are supposed to play a significant role in increasing lung cancer risk. In this study, we aim to investigate the association of IL-6 rs1800795 and IL-1β rs16944 polymorphisms with non-small cell lung cancer (NSCLC) development in the Egyptian population. The study design was composed of 100 NSCLC cases and 100 controls, which were genotyped using the ARMS-PCR technique, electrophoresed on a 2.5% agarose gel, and visualized using ethidium bromide under ultraviolet illumination. The IL-1β rs16944 genotypes were significantly different in NSCLC patients as compared to healthy controls (p = 0.032). Whereas the genotypes and alleles of the IL-6 rs1800795 were not significantly linked to NSCLC incidence (p = 0.726; p = 0.822, respectively). To our best knowledge, this study aimed to uncover the great impact of IL-1β rs16944 genotypes on NSCLC development in the Egyptian population; thus, it may be a gateway for earlier NSCLC prevention.

Keywords: Lung cancer; polymorphism; IL-6; IL-1β; Egypt.

Introduction

Lung cancer stands as the most fatal malignant tumor, which poses a risky health and life conundrum all over the world. Lung cancer has the highest fatality rate and is the second-most commonly diagnosed cancer. In 2020, there were almost 1.8 million lung cancer deaths (18% of all cancer deaths) and approximately 2.2 million new cases (11.4% of total cancers) (Kaanane et al., 2022). In Egypt, lung cancer represents the fastest-growing tumor with a male-to-female ratio of 3.2:1. By increasing the number of women smokers, the incidence rates of lung cancer in women have been elevated (El-Moselhy and Elrifai, 2018).

Among all lung malignancies, NSCLC constitutes 85% and comprises three major types of adenocarcinoma (AC), squamous cell carcinoma (SCC), and large cell carcinoma.
(LCC). Unfortunately, most NSCLC patients are not diagnosed until late-stage (IIIB-IV) disease is present, with a five-year survival rate of only 0%–10% (Duma et al., 2019).

According to epidemiological studies, smoking is the most well-known risk factor for developing lung cancer, accounting for more than 80% of cases (El-Moselhy and Elrifai, 2018). Previous studies demonstrated that the complex combination of chemicals in cigarette smoke causes an inflammatory stress response, producing a continuous source of tumor initiators and promoters in the lung milieu (Landvik et al., 2009). Further research revealed a link between inflammation and tumors, which is largely modulated by various inflammatory cytokines (Tan et al., 2021).

Interleukin-6 (IL-6) is a multifactorial interleukin frequently released by monocytes and macrophages (Dutkowska et al., 2021). It is a powerful cytokine that has both pro-inflammatory and anti-inflammatory properties (Campa et al., 2005). Regarding tumorigenesis, IL-6 functions as an autocrine growth factor for tumors, which directly prevents apoptosis by deleting cell cycle genes (Silva et al., 2017).

The IL-6 gene lies on the short arm of human chromosome 7 (7p21) (Padrón-Morales et al., 2014). The promoter of the human IL-6 gene contains several single-nucleotide polymorphisms (SNPs), among them the IL-6-174G>C (rs1800795), which is the most commonly studied variant (González-Castro et al., 2019). A growing body of research has revealed that IL-6 rs1800795 is linked with increased vulnerability to multiple cancers (Harun-Or-Roshid et al., 2021) as well as the prognosis of several malignancies such as NSCLC, ovarian, bladder, neuroblastoma, and breast cancers (Almolakab et al., 2022; Zhai et al., 2017).

Interleukin-1β (IL-1β) is a member of the IL-1 cytokine family, which is primarily generated by macrophages, monocytes, and lung epithelia (Eaton et al., 2018). It is a pro-inflammatory cytokine that mainly regulates cell proliferation, differentiation, and apoptosis. It has a significant role in enhancing the expression of several inflammatory genes, involving IL-6, IL-17A, and IL-22 cytokines (Li and Wang, 2013).

The IL-1β gene is located on the IL-1 gene cluster on chromosome 2q (2q14-21). Multiple polymorphisms of the IL-1β gene have been recognized (Li et al., 2015). IL-1β-511C>T (rs16944) has been proposed to modulate lung cancer risk, and its functional role has been broadly studied (Zienolddiny et al., 2004). Indeed, the identification of polymorphisms in inflammatory genes has attracted much attention from several researchers, particularly for understanding inter-individual differences in lung cancer susceptibility, risk evaluation, and cancer prevention or detection (Bhat et al., 2014).

Therefore, this study aimed to determine the influence of the two genetic variants IL-6 rs1800795 and IL-1β rs16944 on developing NSCLC in the Egyptian population.

Material and methods

Patients' populations

A total of 200 subjects were recruited for this pilot study, which was divided into 100 primarily diagnosed patients with NSCLC and 100 ethically matched healthy volunteers as controls. The patient group was enrolled on the basis of being over 18, having a confirmed histologic or cytological diagnosis of NSCLC (grades I–III), adequate organ function, measurable disease on computed tomography (stages I–IV), no previous treatment, available clinical data, and no history of cancer or metastatic carcinoma. The absence of a clinical or family history of cancer or pulmonary diseases was necessary to be considered in the control group.

Data collection

Written acceptance was collected from all participants after receiving approval by the Institution Review Board (IRB) of the Faculty of Medicine, Mansoura University, with code number (R.22.06.1736). This research was accomplished pursuant to the Declaration of Helsinki. Using patients’ records, we collected their clinical data, including age, sex, smoking status (nonsmoker and smoker), family history of lung cancer, surgical history, medical history, and symptoms.

Genotyping analysis

Information and details about the SNPs analyzed in our study are represented in Table
1. Primarily, DNA extraction was done for the two cases and control groups. Then, genotyping of the IL-6 rs1800795 was evaluated using the amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method, where three different primers were used: one common and two other allele-specific primers (Elsaid et al., 2014). For the genotyping of IL-1β rs16944, the tetra primer amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) method was performed with two outer primers and two other allele-specific primers (Okayama et al., 2005).

Table 1. Information about the SNPs analyzed in our study population.

<table>
<thead>
<tr>
<th>SNPs ID</th>
<th>Gene</th>
<th>Chromosome</th>
<th>Cytogenetic location</th>
<th>SNP</th>
<th>GMAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800795</td>
<td>IL-6</td>
<td>Chr7: 22727026</td>
<td>7p15.3</td>
<td>-134G&gt;C</td>
<td>0.14</td>
</tr>
<tr>
<td>rs16944</td>
<td>IL-1β</td>
<td>Chr2: 112837290</td>
<td>2q14.1</td>
<td>-511C&gt;T</td>
<td>0.49</td>
</tr>
</tbody>
</table>

SNP single nucleotide polymorphism; Chr chromosome; GMAF global minor allele frequency

The PCR products were electrophoresed on a 2.5% agarose gel and visualized using ethidium bromide under ultraviolet illumination. The PCR products of the IL-6 rs1800795 were observed at 230 bp for the G-allele and the C-allele, as presented in Fig. 1. The IL-1β gene products were identified at 141 bp for the C-allele and 217 bp for the T-allele, as illustrated in Fig. 2.

![Fig. 1 Photomicrograph showing genotyping products of IL-6 (rs1800795) using ARMS PCR. Lanes (1,2), (3,4), (5,6), (7,8), and (11,12) represent common homozygotes (GG); lanes (13,14) represent heterozygotes (GC); lanes (9,10) represent rare homozygotes (CC). Both the G-allele and the C-allele appeared at 230 bp. L 100 bp DNA ladder.](image)

![Fig. 2 Photomicrograph showing genotyping products of IL-1β (rs16944) using ARMS PCR. Lanes (7,8), (9,10), and (11,12) identified heterozygotes (CT); lanes (1,2) and (5,6) identified common homozygotes (CC); lanes (3,4) and (13,14) labeled rare homozygotes (TT). The C-allele is visualized at 141 bp, and the T-allele is visualized at 217 bp. L 100 bp DNA ladder.](image)

Statistics

The IBM Statistical Package for Social Science (SPSS; version 25.0) was used for statistical tests. We processed the qualitative variables as numbers and percentages (N %), which were compared using Fisher’s exact test. Testing for Hardy-Weinberg equilibrium (HWE) was evaluated when the observed and expected genotypic counts of both IL-6 rs1800795 and IL-1β rs16944 were compared among cases and controls by the Chi-square test. The allele and genotype frequencies of IL-6 and IL-1β SNPs were analyzed by Fisher’s exact test. A two-sided P value less than 0.05 indicated a significant association in all statistical tests.

Results

**Characteristics of the study subjects**

Totally, 200 subjects were included in the study, classified as 100 NSCLC patients and 100 healthy volunteers. NSCLC cases included males of 61% and females of 39%, while the control group had males of 84% and females of 16%. Our cases involved 49% smokers and 51% nonsmokers, while the control comprised 28% smokers and 72% nonsmokers. A significant variance was obtained in sex, smoking habits, family history, and medical history between the cases and controls (p < 0.05). The two groups were matched in terms of age and surgical history subgroups (p > 0.05). Detailed clinical data for NSCLC cases and controls are shown in Table 2.
Table 2. Characteristics of non-small cell lung cancer (NSCLC) cases versus controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (n=100)</th>
<th>Controls (n=100)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;55</td>
<td>44</td>
<td>52</td>
<td>0.322</td>
</tr>
<tr>
<td>&gt;=55</td>
<td>56</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>39</td>
<td>16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Male</td>
<td>61</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>49</td>
<td>28</td>
<td>0.002</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>51</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9</td>
<td>0</td>
<td>0.003</td>
</tr>
<tr>
<td>Negative</td>
<td>91</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Surgical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>35</td>
<td>26</td>
<td>0.219</td>
</tr>
<tr>
<td>Negative</td>
<td>65</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>55</td>
<td>38</td>
<td>0.023</td>
</tr>
<tr>
<td>Negative</td>
<td>45</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>38/62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Positive/Negative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspnea</td>
<td>35/65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Positive/Negative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest pain</td>
<td>29/71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Positive/Negative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>7/93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Positive/Negative)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fisher’s exact test; bold values signify p < 0.05.

Association between IL-6 (rs1800795) polymorphism and NSCLC risk

The expected and observed frequencies of IL-6-174G>C (rs1800795) were in accordance with the Hardy-Weinberg equation in the patient and control groups (p > 0.05). The relation between the IL-6 rs1800795 variant and NSCLC occurrence was evaluated, as presented in Table 3. The results showed a low frequency of the rare genotype ‘CC’ (1%) among NSCLC patients and its absence among controls (0%), with a non-significant variance of the genotypes between NSCLC patients and controls (p = 0.726).

Association between IL-1β (rs16944) polymorphism and NSCLC risk

When IL-1β-511C>T (rs16944) frequencies were tested for genetic equilibrium using the Hardy-Weinberg law, they significantly deviated from expected frequencies (p < 0.001) in both cases and controls. This significant deviation may be attributable to the higher frequency of the protective heterozygote that may be related to the Egyptian population.

Table 3. Distribution of IL-6 (rs1800795)-174G>C polymorphism among Egyptian patients with (NSCLC) compared to controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n)</th>
<th>Controls (n)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>78 (78%)</td>
<td>81 (81%)</td>
<td>0.726</td>
</tr>
<tr>
<td>GC</td>
<td>21 (21%)</td>
<td>15 (15%)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>177 (88%)</td>
<td>181 (90.5%)</td>
<td>0.822</td>
</tr>
<tr>
<td>C</td>
<td>23 (11.5%)</td>
<td>19 (9.5%)</td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X²= 0.08</td>
<td>X²= 1.1</td>
<td>P= 0.965</td>
<td>P= 0.3</td>
</tr>
</tbody>
</table>

Fisher’s exact test; HWE Hardy-Weinberg equilibrium

As shown in Table 4, the genotypic and allelic statistics of the IL-1β rs16944 variant among NSCLC patients and controls were established. The results of rs16944 genotypes revealed a significant difference in NSCLC patients other than healthy controls (p = 0.032).

Table 4. Distribution of IL-1β (rs16944)-511C>T polymorphism among Egyptian patients with (NSCLC) compared to controls.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Cases (n)</th>
<th>Controls (n)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>10 (10%)</td>
<td>2 (2%)</td>
<td>0.032</td>
</tr>
<tr>
<td>CT</td>
<td>86 (86%)</td>
<td>96 (96%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>4 (4%)</td>
<td>2 (2%)</td>
<td></td>
</tr>
<tr>
<td>allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>106 (53%)</td>
<td>100 (50%)</td>
<td>0.777</td>
</tr>
<tr>
<td>T</td>
<td>94 (47%)</td>
<td>100 (50%)</td>
<td></td>
</tr>
<tr>
<td>HWD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X²=52.7</td>
<td>X²=84.6</td>
<td>P&lt; 0.001</td>
<td>P&lt; 0.001</td>
</tr>
</tbody>
</table>

Fisher’s exact test; HWE Hardy-Weinberg equilibrium; Bold values express the p < 0.05.

Discussion

Cytokines are a group of functional proteins that are induced in both inflammatory cells and cancerous cells. They have gained considerable attention, particularly for being important orchestrators of cancer-inflammation interactions. It has been shown that some inflammatory cytokines are crucial for continuing inflammatory conditions, modulating the transition to malignant epithelial cells, hindering host immune surveillance, and stimulating tumor growth and spread (Bai et al., 2013). In light of the growing research that focuses on the etiologic role of
inflammation in lung malignancies, we conducted this study to consider the possible impact of polymorphisms in inflammatory genes, such as \textit{IL-6} (rs1800795) and \textit{IL-1β} (rs16944), on NSCLC development in Egyptian subjects.

This study demonstrated that males have a higher incidence of NSCLC than females. Our results were in line with evidence from epidemiological studies, which revealed that more men than women smoke tobacco and have higher rates of incidence and mortality. However, non-smoking women are more vulnerable to evolving lung cancer than non-smoking men, and females with NSCLC have higher rates of EGFR mutations and the prevalence of adenocarcinomas with lepidic features (de Groot et al., 2018).

In addition, the study revealed other risk factors associated with the incidence of NSCLC, including smoking habits, family history, and other comorbidities. As evidenced in many reports, they are the most brilliant and independent risk factors that can affect the pathogenesis, outcomes, and prognosis of lung cancer (El-Moselhy and Elrifai, 2013).

This study identified that there was a non-significant variation between the \textit{IL-6} rs1800795 polymorphism and NSCLC susceptibility. Several studies were in line with our findings. A report by Gao et al. (2020), revealed no genotypic distribution differences for \textit{IL-6} rs1800795 in lung cancer patients using meta-analysis. Eaton et al. (2018) conducted a nested case-control study, comprising 625 cases and 625 matched controls, which revealed a non-significant correlation between genotypes of the \textit{IL-6} rs1800795 variant and lung cancer risk (\(P = 0.97\), for genotypes). Also, Liu et al. (2015) established a meta-analysis study that showed that genotypic and allelic frequencies of the \textit{IL-6} rs1800795 were not statistically associated with lung cancer susceptibility. A comprehensive meta-analysis showed that rs1800795 was generally linked to the cancer risk of both Africans and Asians via different inherited models. However, the stratifying analysis based on cancer subtypes revealed no significant relationship with lung cancer, which may be due to heterogeneity (Harun-Or-Roshid et al., 2021). This was observed in Asians, who have lower or even absent \textit{IL-6}-174C allele frequencies than Caucasians (Tian et al., 2015).

Our results were inconsistent with those of Kaanane et al. (2022) who studied the effect of the \textit{IL-6} (rs1800795) SNP on 150 lung cancer cases and 150 controls in the Moroccan population. The study revealed a significant difference in \textit{IL-6} (rs1800795) genotypes and alleles between cases and controls. In addition, a meta-analysis study has confirmed that the \textit{IL-6} rs1800795 has a significant linkage with elevated lung cancer incidence (\(P = 0.003\), for alleles) and in Caucasian and Asian populations (\(P < 0.001, P = 0.003\), for alleles, respectively) (Peng et al., 2018).

On the other hand, the current study observed a significant difference in \textit{IL-1β}-511 frequency between NSCLC and healthy Egyptian subjects. In agreement with our results, Zienolddiny et al. (2004) confirmed that the \textit{IL-1β} polymorphisms were markedly linked to lung cancer susceptibility in the Norwegian population. The study included 251 lung cancer cases and 271 healthy volunteers, which were largely different among \textit{IL-1β}-511 genotypes and allotypes. The nested case-control study of Eaton et al. (2018) conducted on contributors from the \(\beta\)-Carotene and Retinol Efficacy Trial discovered that the rs16944 variant was associated with the lung cancer incidence (\(p = 0.03\)). A recent meta-analysis was designed to explore the impact of 11 different variants of \textit{IL-1β}, \textit{IL-4}, \textit{IL-6}, \textit{IL-8}, and \textit{IL-10} from 43 studies on lung cancer susceptibility. The study selected 12 case-controlled studies for the rs16944 SNP and indicated that its allelic and genotypic frequencies were significantly linked to lung cancer susceptibility (Ding et al., 2021). On the contrary, other studies by Li and Wang, Pérez-Ramírez et al., and Kiyohara et al. (2013; 2017; 2014) showed that there was a non-significant difference in the rs16944 variant between lung cancer cases and controls.

Thus, the overall findings suggest the significance of the \textit{IL-1β} rs16944 polymorphism in NSCLC occurrence. However, the study comprised limited samples collected from a single center. Therefore, it is important to design further studies in other ethnic populations with larger sample sizes to support our findings.

**Conclusion**

In conclusion, this pioneering study illustrated the association between \textit{IL-1β}-511C>T polymorphism and NSCLC in
Egyptian subjects. Our work indicated that the IL-1β-511C>T genotypes were significantly different in NSCLC compared to the healthy volunteers. However, the IL-6-174G>C SNP was not significantly associated with the development of NSCLC. This study shed light on the role of IL-1β gene polymorphism in the earlier prevention of developing lung cancer as a candidate genetic marker.

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Declaration of competing interest

All authors declare that there is no conflict of interest between them.

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3 وحدة الوراثة، مستشفى الأطفال، جامعة المنصورة، المنصورة، مصر

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