An Investigative Study of Smoking Effects on IL-31 Levels and Leukocyte differential counts in Humans

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ABSTRACT
Interleukin 31 is a helix inflammatory cytokine that has been recently discovered. It belongs to the IL-6 family. About 1/3 of the world population smoke cigarettes. Therefore, it involved the progression and pathogenicity of Chronic Obstructive Pulmonary Disease (COPD) as well as in asthma. This study aimed to evaluate IL-31 levels in smokers and non-smokers individuals and its correlation to differential WBCs count. Peripheral blood from the vein of smokers (n=40) and non-smokers healthy persons (n=30) was taken. One part of each sample was used for WBCs differential count, while the other was used to obtain serum for evaluating IL-31 levels using the ELISA kit. These findings showed a significant elevation in the blood eosinophilic count, and eosinophilia was common in 40% of smokers. A non-significant increase in both lymphocytes and neutrophils count was found whereas monocytes were decreased non-significantly in smokers compared to non-smokers. The basophilic count did not express any changes in study subjects. The serum level of IL-31 showed a high but not significantly increased in smokers’ individuals. Smoking cigarettes lead to an increase the allergic inflammatory markers such as eosinophils, lymphocytes, and IL-31 which, suggesting a remarkable role of IL-31 in the conditions resulting from smoking cigarettes.

Keywords: Smoking, IL-31, Eosinophils, lymphocytes, Neutrophils


INTRODUCTION
Interleukin 31 (IL-31) is a helix inflammatory cytokine that has been recently discovered. It belongs to the gp130 / IL-6 family, which includes other cytokines such as IL-6. This interleukin secreted by T-cells (CD4+ T cells), and mast cells [1, 2] and the gene that encodes human IL-31 is located on chromosome 12q24.31. The IL-31 plays an important role in inflammatory diseases such as atopic dermatitis [3, 4], inflammatory bowel disease, and hypersensitivity[5, 6]. Many studies linked IL-31 to rhinitis, asthma, and dermatitis [7]. For this reason, it has been believed that suppression of IL-31 may be a useful way to treat inflammatory diseases.
The receptor of IL-31 consists of two subunits, IL-31 receptor alpha, also known as IL-31RA and oncostatin-M receptor beta or OSMR. The mRNA expression of the alpha receptor is found in various tissues, such as the testis, bone marrow, and skin. Moreover, it is expressed in different cells, such as activated monocytes (macrophages), dendritic cells (DCs), eosinophils, basophils, and keratinocytes, while OSMR mRNA is broadly expressed in many tissues[7-9].
Smoking cigarettes is a prevalent habit worldwide, affecting about 1/3 of the world’s population [10]. This habit is crucial for the progression and pathogenicity of Chronic Obstructive Pulmonary Disease (COPD) [11, 12] and asthma [13, 14]. Several chemical substances within tobacco are toxic, including polycyclic aromatic hydrocarbons (benzo[a]pyrene), N-nitrosamines, heavy metals (arsenic, cadmium, chromium, and nickel), alkaloids (nicotine and its metabolite- cotinine) and aromatic amines. The pathogenicity from smoking leads to various disorders, including inflammation, immune modulation, oxidative damage, endothelial dysfunction, and genetic and epigenetic alterations. Many studies have explored this topic, but the effect of tobacco is still not fully understood [15-17]. This study aimed to evaluate IL-31 levels in smokers and non-smokers and its correlation with differential white blood cell (WBC) counts.

**Materials and Method**

**Study subjects and sample collection**
Male healthy subjects aged 25-45 yrs., both smokers (n=40) and non-smokers (n=30) are eligible for the study, with smokers being considered as the study group while non-smokers act as controls. The sample collection period was from September to December 2019. The type of smoking included only cigarettes, only Shisha, or both cigarettes and Shisha. Information about all participants was collected through self-reporting questionnaires. The blood collection was performed aseptically through sterilization of equipment and skin cleaning. Blood was collected from superficial vein puncturing using sterile syringes. The collected blood samples were divided into two parts: the first part was placed in EDTA tubes for complete blood count and differential count tests, while the other part was placed in Gold-top SSG tubes to separate the serum for ELISA. The serum samples were stored at -20°C until analysis.

**Complete Blood Count**
Blood films were made from anticoagulated blood by placing a drop of blood on a clean smooth slide according to the classical method [18]. The blood films were stained with Leishman's stain, examined under the light microscope, and the differential WBCs count was performed to record the percentages of each WBC type.

**Determination of Interleukin-31**
Sera from all study subjects were used to estimate the level of IL-31 using ELISA kit according to the manufacturer protocol (Cusabio, USA). The concentration of IL-31 was calculated using a standard curve created by the standard protein provided by the kit.

**Statistical analysis**
The data was statistically analyzed using a normality test at the beginning followed by parametric (t-test) and non-parametric test (Mann-Whitney U test) in MINTAB program.

**Results**

**Differential WBCs count in smokers**
The results of the differential WBCs count showed that smokers had a significant elevation in eosinophils number compared with the control group, and 40% (16/40) of smokers had eosinophilia. Neutrophils and lymphocytes showed a non-significant increase in smokers while monocytes showed a non-significant decrease in number in smokers, no changes in the number of basophils were noticed. The count of WBCs is shown in Table 1 and Figure 1 A, B, C and D.
Table 1 Differential leukocytes count in study subjects compared with the control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Smoker (Mean ± SE) X10^7/L</th>
<th>Non-smokers (Mean ± SE) X10^7/L</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>4.115±0.242</td>
<td>3.975±0.410</td>
<td>0.952</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.320±0.035</td>
<td>0.145±0.023</td>
<td>0.002</td>
</tr>
<tr>
<td>Basophils</td>
<td>0±0.004</td>
<td>0±0.005</td>
<td>0.572</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2.644±0.115</td>
<td>2.578±0.102</td>
<td>0.670</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.245±0.019</td>
<td>0.270±0.028</td>
<td>0.817</td>
</tr>
</tbody>
</table>

Figure 1. The count of WBCs types in smokers and non-smokers. A. Eosinophils were significantly elevated in smokers in comparison with nonsmokers control (p= 0.002). B. Lymphocytes were not significantly increased in smokers compared with nonsmokers control (p= 0.670). C. Neutrophils were not significantly elevated in smokers compared with nonsmokers control (p=0.952). D. Monocytes were not significantly decreased in smokers compared with nonsmokers control (p=0.817).

Serum level of IL-31 in smokers
The results of IL-31 estimation in the sera of smokers showed a high level but not significant elevation in comparison with its level in nonsmokers control (109.944±64.434pg/ml and 60.723±5.40pg/ml, respectively) and the P value was 0.46 as shown in Figure 2.
DISCUSSION

The current study investigated the effect of smoking on innate immunity represented by complete blood count and the adaptive immunity expressed as the level of IL-31 in smoker and non-smoker persons. The present results showed that eosinophils are significantly higher in smokers, and the lymphocytes also were elevated but not significantly. The elevation of WBCs in the smoker individuals was documented previously[19]. It has reported that the changes in the number of eosinophils are associated with a minor negative impact on lung function in both smokers and non-smokers[20]. In patients suffering from mild to severe COPD, it has found that eosinophilia increases the risk of disease exacerbation[21]. The eosinophils in the sputum of former and current smokers found to be in a high concentration that made it a good biomarker to classify COPD patients in comparison with eosinophils high concentration in theirblood [22]. In this regard, Pathak et al., (2014) [23]pointed out the increase of both eosinophil and leucocytes count in blood of smoker subjects in India. Furthermore, in allergic asthma, the increased eosinophils considered as a characteristic feature [24] eosinophils counts of 290-400 cells/μl have been associated with progression of asthma [25]. Additionally, the increase in eosinophils levelin different duration of smoking is linked to both COPD and asthma progress [21]. The Smoking is strongly linked to elevated WBCs and, ceasing smoking led to normal WBCs count in a period of year time, which makes it a reversible change[19, 26, 27]. The most likely explanation for the increased leukocytes in smokers' blood is the ability of nicotine to induce releasing of catecholamine and steroid hormones from the core of the adrenal gland. Wherein they described the increase in the level of certain endogenic hormones, like epinephrine and cortisol, leading to an increase in the number of white blood cells[28, 29]. In another study, smoking led to the activation of the aryl hydrocarbon receptor (AHR) pathway, which is important in environmental pollutant metabolism. The activation of AHR can cause many diseases, including inflammatory diseases like arthritis as it enhances the development of Th17 directly [30]. The knowledge of how smoking affects neutrophils is still limited but, a study reported that total particulate matter from cigarette smoking leads to neutrophil reprogramming and they behaved as immune suppressor by reducing both STAT1 activations and NADPH oxidase of inducible nitric oxide synthase (iNOS) [31].

Regarding the rest of the WBCs (monocytes and Basophils) which showed non-significant decrease in comparison with non-smokers, there is an investigation that reported an increase in eosinophils and lymphocytes counts in smokers, while the other WBCs was not affected, this suggests that the influence of smoking on WBCs differential count can be affected by other factors such as smoking behavior, the duration of smoking, race, sex, and age[32]. Moreover, smoking can induce monocytes dysfunction according to the mentioned factors [33]. The IL-31 was not significantly raised in smokers in the current study. The role of IL-31 during smoking still unknown as this
interleukin was recently introduced. Many studies investigated the role of IL-31 in several inflammatory disorders. In asthma, recent evidence indicated the involvement of IL-31 in promoting allergic inflammations and it also contributed to the airway epithelial responses [6, 34, 35]. In another work, a positive correlation found between IL-31 serum levels, IL-5, IL-13, and thymic stromal lymphopoietin (TSLP) which are Th2 cytokines and asthma severity. This study proposed an important role of IL-31 in asthma pathogenicity [36]. Recently, several studies proposed the correlation between the couple novel cytokines, IL-31, and IL-33 and described them as IL-31/IL-33 axis, as well they proved their association in many inflammatory disorders [37-39]. Since IL-31 is relatively newly discovered, no information available concerning the effects of smoking on its levels and functions, but this study showed that smoking leads to the elevation of serum IL-31 in smoker individuals compared to non-smoker subjects suggesting there is a role of IL-31 in any inflammatory disorder triggering by cigarette regardless of the type of smoking. One limitation of this study, all participants were male, there were few numbers of smokers women owing to smoking as a habit is not quite acceptable and desirable in our society. Similar findings were documented by previous investigations in other societies [40-42].

CONCLUSION
This paper investigated the impact of smoking on interleukin -31 level and differential counts of leucocytes in the blood of smokers individuals compared to nonsmoker ones. The main conclusion that can be drawn based upon the results obtained from this study was the noticeable elevation of both IL-31 level and eosinophil in blood of smoker persons. As well as, the rest of leucocytes expressed a non-significant decrease in number in the blood of smokers, except lymphocytes that recorded an increased number but non- significantly differed from that of the control group. Smoking cigarettes increased the allergic inflammatory markers such as eosinophils, lymphocytes, and IL-31, which suggests a remarkable role of IL-31 as pro-inflammatory cytokines inducer. The early detection of its level could help prevent the development of several inflammation-related diseases.

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Contribution of Authors
The first author: designed and supervised research methodology, Manuscript final reading.
The second author: conceived the idea, Manuscript writing.
The third author: Specimens collection, Performed experiments.
The fourth author: Specimens collection, Performed experiments.

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