

Correlation between Genotoxicity and Interleukin-6 in Smokers: A Rodent Model

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ABSTRACT

Objective: To investigate the relation between genotoxicity and interleukin-6 in rats exposed to cigarette smoke.

Study Design: An experimental study.

Place and Duration of Study: Army Medical College, Rawalpindi and Armed Forces Institute of Pathology, Rawalpindi, in 2016.

Methodology: Seventy healthy Sprague Dawley rats were placed in smoke chambers at animal house of National Institute of Health, Islamabad. Cigarette smoke was given to them for 3 months. Genotoxicity was assessed by Cytokinesis Block Micronucleus (CBMN) assay. Enzyme linked immunosorbent assay (ELISA) kit was used to determine Interleukin-6 in study samples. Pearson correlation was used to find the correlation between genotoxicity and IL-6.

Results: The mean IL-6 and micronuclei frequency was 49.48 ± 19.69 ng/L and 6.77 ± 0.73 , respectively. Weak positive association was found between micronuclei frequency and IL-6 in smoke exposed rats ($r=0.266$, $N=70$, $p=0.026$).

Conclusion: Genotoxicity and inflammation are associated in smokers. The present study concluded that smoke exposure elicited a proinflammatory profile, which might have promoted DNA damage in smokers.

Key Words: Cigarette smoking, IL-6, DNA damage, Rats.

INTRODUCTION

Cigarette smoking is the chief cause of avoidable morbidity and mortality. Cigarette smoke contains considerable adverse chemicals. These biohazards contribute to inflammation, oxidative stress and DNA damage.¹

Cigarette smoking is coupled to various health issues, including cancers, pulmonary and cardiovascular disorders;² therefore, its toxicity is required to be evaluated. Raised inflammation and genotoxicity in smokers may be responsible for its destructive health impacts.³

Genotoxicity happens frequently, but DNA repair pathways in the cell generally repair it. In case of failure of DNA repair pathways, cells experience cell death or accumulate mutations in somatic cells, which lead to cancer development.⁴ Genotoxicity is produced by environmental exposure to genotoxins and lifestyle factors like alcohol, smoking, drugs and stress. Genotoxicity can lead to multiple diseases, such as neurodegenerative disorders, cancers, immune deficiencies, infertility and even aging.⁵ Genotoxicity is

thought to be one of the mechanisms by which cigarette smoke initiates disease.⁶

Micronucleus assay has been found to be an excellent tool to serve as a genotoxicological biomarker. Micronuclei (MN) are tiny fragments that appear in the cell cytosol during cell division as a result of chromosomal damage.⁷

Interleukin-6 (IL-6) is a multifunctional cytokine involved both in the beneficial acute inflammatory response and in the detrimental chronic low-grade systemic inflammation.⁸ IL-6 has two different pathways for its induction of intracellular signaling: classic signaling and trans-signaling.⁹ In the classic pathway, IL-6 binds the membrane-bound IL-6 receptor, located on the surface of hepatocytes and some leukocytes, and activates the IL-6 classic signaling transduction cascade with the homodimerization of the membrane-bound β -receptor glycoprotein 130.¹⁰ In the trans-signaling axis, circulating IL-6 forms a heterodimer with the soluble form of IL-6 receptor, that could transduce a proinflammatory cascade in virtually any cell types through direct binding with membrane-bound gp130.¹¹ Classic signaling is mainly responsible for the beneficial regenerative and antibacterial effects of IL-6,¹² while the trans-signaling seems to account for the majority of the deleterious effect of IL-6.¹³

Cigarette smoke can provoke inflammation through stimulation of inflammatory cells which produce pro-inflammatory cytokines. Chronic inflammation is broadly recognized to generate cancer development through genotoxicity.¹⁴ However, little is known about the correlation between the IL-6 and DNA damage in smokers.

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This study, therefore, aimed to investigate the relationship between inflammation and DNA damage in smokers.

METHODOLOGY

It was an experimental study carried out at Army Medical College, Rawalpindi and Armed Forces Institute of Pathology (AFIP), Rawalpindi in 2016, after approval by Ethical Committee of Army Medical College. Seventy healthy Sprague Dawley rats were procured in the animal house of NIH, Islamabad. Healthy Sprague Dawley rats weighing 220 ± 30 grams and age between 6-8 weeks were included in the study. Diseased rats at the time of study were excluded from the study.

The animals were placed in smoke chambers and supplied with pelleted food and water *ad libitum*. Cigarette smoke was given to rats for four hours per day for five days a week for 12 weeks.

At the end of 12 weeks, 6-7 ml blood was collected from each rat. Three ml was poured into the vacuum tubes containing clot activator. It was centrifuged and serum obtained was stored at -80°C . ELISA kit was used to measure IL-6 in the study samples.

Three ml blood was taken into the lithium heparinized tubes for estimation of micronucleus frequency by cytokinesis block micronucleus (CBMN) assay to assess genotoxicity. Lymphocyte cultures were performed. Frequency of micronuclei, was quantified in 1000 lymphocyte nuclei from each sample. The micronuclei were of different sizes, and before being accepted as micronuclei they were compared with the main nucleus in terms of size, structure, color, and number.

SPSS version 17 was used to analyse the data. Mean and standard deviation (SD) was calculated for IL-6 and MN frequency. Correlation between IL-6 and MN frequency was determined by Pearson correlation test. A p-value < 0.05 was considered statistically significant.

RESULTS

The correlation between mean of Interleukin-6 and micronuclei frequency in smoke exposed rats. The mean IL-6 and micronuclei frequency was 49.48 ± 19.69 ng/L and 6.77 ± 0.73 , respectively.¹⁵ Statistically significant weak positive correlation was found between IL-6 and micronuclei frequency ($r=0.266$, $N=70$, $p=0.026$).

DISCUSSION

Cigarette smoking and tobacco chewing are common modes of consuming tobacco.¹⁶ Cigarette smoke, a major public hazard, is a detrimental blend of numerous compounds which may induce carcinogenesis by their mutagenic and genotoxic effects.¹⁷ Cigarette smoke also contains many oxidants and free radicals that induce oxidative damage.¹⁸ Oxidative stress induced by

tobacco smoking is one of the main causes of DNA damage and is known to be involved in the development of several cancers.¹⁹

Cigarette smoke causes oxidative damage in DNA, either directly or through generation of reactive oxygen species as evidenced by human and animal studies.²⁰ Tobacco exposure is the leading cause of cancers involving the oral cavity, conductive airways, and the lung.²¹

Wolz *et al.* carried out his study to explore the genotoxic effects of sidestream cigarette smoke at different concentrations using the alkaline comet assay. Human bronchial epithelial cells were exposed to sidestream smoke or fresh air for 1 hour. Results showed that sidestream cigarette smoke exposure causes significant DNA strand breaks.²²

IL-6 is a cytokine with a multifactorial function and induces both pro- and anti-inflammatory responses.²³ Cigarette smoke exposure induces inflammation in both humans and mice.²⁴

Conflicting results were reported by Koczulla *et al.* He conducted a study to show the effect of smoking on inflammatory mediator, IL-8. Twenty-nine smokers and 19 non-smokers were enrolled in the study. IL-8 levels were determined by ELISA in each participant. Results showed no significant change in the level of IL-8 between the groups.²⁵

In cigarette smokers, *p53* gene is commonly mutated. This gene is a tumor suppressive gene, so mutations affecting *p53* gene lead to unchecked cellular growth and tumor formation. Many studies have illustrated that cigarette smoke induces DNA-strand breaks in mammalian cells, and sister chromatid exchanges are shown in bone marrow and lung cells exposed to cigarette smoke indicating its genotoxic effect. Moreover, increased trend of mutations, translocations and DNA strand breaks have been found in newborns of smoking mothers in humans. Genotoxicity induced by cigarette smoke is employed not only through direct damage to DNA but also through indirect mechanisms; for example, chronic inflammation and production of reactive oxygen species.²⁶

The results of this study showed that plasma proinflammatory cytokine, IL-6, was elevated in the smoke exposed rats. Micronuclei were also enhanced in plasma of exposed rats, indicative of DNA damage. Inflammation is associated with increased DNA damage; whereas, the link between inflammation and genotoxicity is still obscure in smokers. To the best of authors' knowledge, no study in Pakistan has yet determined the relationship between IL-6 and micronuclei frequency in smokers. This study determined correlation between IL-6 and micronuclei frequency and results showed significant positive association, thus linking inflammation and genotoxicity in smokers.

CONCLUSION

The present study concluded that smoke exposure elicited a proinflammatory profile which might have promoted DNA damage in smokers. However, further studies are needed to probe into the details of mechanisms associated with these findings.

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