STM JOURNALS

Research & Reviews: A Journal of
Immunology

ISSN: 2277-6206 (Online)
ISSN: 2349-1280 (Print)
Volume 11, Issue 1, 2021
DOI (Journal): 10.37591/RRJoI

http://medicaljournals.stmjournals.in/index.php/RRJoI/index

Research RRJoI

Effect of Nicotine on Cytokine Production by Human Mononuclears: Perspective of COVID-19

Waghmode Meghmala S.[1], Divekar Akshay B.[2], Dhasade Akshay Y.[3], Mulani Mansurali K.[4], Patil Neha N.[5,*]

Abstract

Nicotine is present in the tobacco-containing products such as cigars, cigarettes, chewing tobacco, snus (an oral tobacco) and pipe tobacco. In Indian population, nicotine containing products are consumed by the 57% of population either in chewing form or smoking form. The correlation of nicotine and lung cancer in chronic smokers as well as nicotine consumption and COVID-19 incidence has to be justified. Hence current research was carried to study the effect of nicotine on cytokine production by peripheral blood mononuclear cells (PBMC's). MACSPlex Cytokine12 assay was used for the estimation of cytokine after treatment of PBMC's with the nicotine. Concentration of cytokines (IL 10, IL 12p70, IL 17, IFN α, IFN γ, GM CSF, IL 4, IL 5, IL 2 and IL 9) was found to be increased in the sample containing PBMC's treated with the 20 µl of nicotine indicating that nicotine promotes PBMC's for the secretion of IL 10, IL 12p70, IL 17, IFN α, IFN γ, GM CSF, IL 4, IL 5, IL 2 and IL 9 but it impedes the production of IL 6 and TNF α the important pro-inflammatory cytokines. IL 6 and TNF α are the important pro-inflammatory cytokines in COVID-19 infection being responsible to switch the infection from mild to a fatal one. The impeding characteristics of nicotine can be proposed to have potential of pharmaceutical nicotine as a future treatment option in COVID-19. The detailed studies are needed for developing nicotine patches as a prospective cytokine release syndrome (CRS) therapy for COVID-19 to combat this dreadful pandemic.

Keywords: Cancer, COVID-19, cytokine, nicotine, immunity

INTRODUCTION

Consumption of nicotine containing products by small children and aged people is global concern due to its side effects on health. Higher nicotine content has been reported in smoking form of tobacco as compared to chewing form [1]. Absorption of nicotine occurs both through the buccal mucosa and the gastrointestinal tract in tobacco chewers whereas absorption of nicotine occurs primarily through the pulmonary vasculature in tobacco smokers.

During chewing of tobacco, the nicotine in tobacco preparations remains in contact with oral mucosa for long period of time and absorbed by the tobacco chewer. Hence even though the content of nicotine is less than the smoke forms, it is responsible for carcinogenic effects. In saliva the tobacco-specific N-nitrosamines are extracted easily and there is increase in absorption in alkaline conditions in case of tobacco chewers as compared to smokers.

Pandemic situation posed due to SARS-CoV-2, leads to find the strategies to control cytokine release

***Author for Correspondence**
Patil Neha N
E-mail: nehanitinpatil@gmail.com

[1]Assistant Professor, Department of Microbiology, P.D.E.A.'s Annasaheb Magar Mahavidyalaya, Hadapsar, Pune, Maharashtra, India
[2-4]Research Scholar, Department of Microbiology, P.D.E.A.'s Annasaheb Magar Mahavidyalaya, Hadapsar, Pune, Maharashtra, India
[5]Professor and HOD, Department of Microbiology, P.D.E.A.'s Annasaheb Magar Mahavidyalaya, Hadapsar, Pune, Maharashtra, India

Received Date: December 31, 2020
Accepted Date: February 18, 2021
Published Date: April 16, 2021

Citation: Waghmode Meghmala S., Divekar Akshay B., Dhasade Akshay Y., Mulani Mansurali K., Patil Neha N. Effect of Nicotine on Cytokine Production by Human Mononuclears: Perspective of COVID-19. Research & Reviews: A Journal of Immunology. 2021; 11(1): 4–7p.

syndrome (CRS) by blocking the signal transduction pathway. To manage inflammation, the scientists propose to use nicotine as an exogenous α7nAchR agonist. Epidemiological evidence based on clinical SARS-CoV-2 studies in China, researchers has made hypothesis about the potential CRS therapy using nicotine [2]. Negative correlations between smoking and corona infections attributed to control of cytokine release due to nicotine [2] and inhibition of Furin [3, 4].

Nicotine has been reported in the treatment of autoimmune diseases due to its protective and anti-inflammatory effects, particularly by binding to α7 subunit of nicotinic acetylcholine receptors [5].

Current research was done on testing the effect of nicotine on cytokine production by PBMC's isolated from healthy volunteer who will form the basis of less prevalence of COVID-19 among smokers.

MATERIALS AND METHODS

Isolation of Peripheral Blood Mononuclear Cells

Venous blood was drawn from healthy person with his consent. Blood was collected into 10 mL EDTA tubes, after which peripheral blood mononuclear cells (PBMCs) were isolated as described. In short, blood was diluted in phosphate buffered saline (PBS) (1:1) and fractions were separated by Ficoll (HiSep LSM LS001) density gradient centrifugation. Cells were washed twice with PBS and re-suspended in RPMI-1640 (Gibco, Invitrogen, Breda, and The Netherlands) (RPMI-1640 Dutch modification supplemented with 10 µg/mL antibiotic antimycotic). The PBMCs were counted using a neubauer chamber and were plated in 96 well round-bottom plates (Moxcare, USA) at a final concentration of 1×10^6/mL, in a total volume of 100 µL as per the method of Mallone et al. [6]. The PBMCs were treated with nicotine and one set was kept as control.

Effect of Nicotine on Cytokine Production

The influence of nicotine on cytokine production by PBMCs was tested. Incubation of 20 µl of nicotine was done with 0.5 mL of PBMC (4×10^6/mL). One set was kept as control. A humidified atmosphere containing 5% CO_2 was employed for 24 h at 37°C to maintain the cultures. The cells were removed by centrifugation at 250 g for 10 min at the end of the incubation period. The supernatants were collected and assayed for cytokines content. They were preserved at -70°C.

MACSPlex Cytokine 12 Assay

The MACSPlex Cytokine 12 Kit (Miltenyi Biotec GmbH) was employed for analyzing harvested cell free supernatants after 24 h. To both the unknown samples and to serial dilutions of the MACSPlex cytokine 12 standard the MACSPlex cytokine 12 capture beads were added. By using the MACSPlex capture beads, the cytokines were captured during a 2-hour incubation period. To form sandwich complexes during a 1-hour incubation period, the MACSPlex cytokine 12 detection reagent containing a mixture of 12 APC-conjugated anti cytokine antibodies were added. For estimation of each of 12 cytokines, standard curves were used. The concentration of each cytokine in the unknown samples is estimated from the medium of the APC fluorescence of each capture bead population. Bead populations constitutes of 12 types that have been coated with capture antibodies specific for following cytokines: GM-CSF, IFN-α for MACSPlex cytokine 12 capture beads. The different fluorescence intensities detected in the FTIC (B1) and PE (B2) channel of the MACSQuant® Analyzer and MACSQunt Analyzer distinguish the bead population as per instructions given in manual.

RESULTS AND DISCUSSION

Researchers are trying to find the effect of nicotine on the immune functioning of peripheral blood mononuclear cells as well as cancer cell line. Nicotine has been reported to have immunomodulatory activity on bone marrow-derived monocytes/macrophages by limiting the concentration of proinflammatory cytokines TNFα, IL-1β, IL-6 and IL-12 and stimulating anti-inflammatory cytokines like IL-10 and TGF-β [7]. In case of the infection by Corona virus, there are severe changes in cytokine responses in patients [8]. Merad and Martin studied pathological roles of macrophages in COVID-19 infection

[9]. They selected a platform that monitors interleukin (IL)-6, IL-8, IL-1β and tumor necrosis factor (TNF), the well-established targets for anti-inflammatory therapeutics. The testing of 1,500 patients on the day of hospitalization was done. The correlation of the serum cytokine concentrations with disease outcome was studied. It was found out that IL-6, IL-8 and TNF, and to a lesser extent, IL-1β, were elevated at the time of hospitalization. The proinflammatory cytokines remained elevated throughout the disease course unless patients were treated with steroids or remdesivir, which reduced the level of circulating IL-6. It was detected that IL-6 and TNF are regulated independently and hence can be aimed in parallel to diagnose and treat the patients with severe disease. The patients with severe disease had increased IL-6 and other proinflammatory cytokines, while those with moderate disease had a pattern that suggested T cell priming. The findings of the research study by Chen et al. also proved that higher levels of IL-2R, IL-6, and TNF-α are present in COVID-19 patients with pneumonia as compared to COVID-19 patients without pneumonia [10]. The severity of lung injury accessed by CT severity score and PaO2/FiO2 were statistically correlated with both IL-2R and IL-6 in COVID-19 patients with pneumonia.

The less prevalence of viral infection in smokers is attributed to anti-inflammatory and immunomodulatory activity of nicotine by researchers [2, 11, 12].

Continuous efforts are being put in to find out the effect of nicotine on the immune functioning of peripheral blood mononuclear cells as well as cancer cell line. Nicotine has been reported to have immunomodulatory activity on bone marrow-derived monocytes/macrophages by limiting the concentration of proinflammatory cytokines TNFα, IL-1β, IL-6 and IL-12 and stimulating anti-inflammatory cytokines like IL-10 and TGF-β [7]. Concentration of cytokines (IL 10, IL 12p70, IL 17, IFN α, IFN γ, GM CSF, IL 4, IL 5, IL 2 and IL 9) was found to be increased in the sample containing PBMC's treated with the 20 µl of nicotine indicating that nicotine promotes PBMC's for the secretion of IL 10, IL 12p70, IL 17, IFN α, IFN γ, GM CSF, IL 4, IL 5, IL 2 and IL 9 but it impedes the production of IL 6 and TNF α the important pro-inflammatory cytokines (Table 1). IL 6 and TNF α are the important pro-inflammatory cytokines in COVID-19 infection being responsible to switch the infection from mild to a fatal one. The impeding characteristics of nicotine can be proposed to have potential of pharmaceutical nicotine as a future treatment option in COVID-19. The detail studies are needed for developing nicotine patches as a prospective cytokine release syndrome (CRS) therapy for COVID-19 to combat this dreadful pandemic.

Table 1. Estimation of Cytokine Production by PBMC's treated with 20 µl of Nicotine and Control.

Name of the cytokine	**Control (pg/ml) ± SEM**	**Secreted cytokine by PBMCs treated with Nicotine (pg/ml) ± SEM**	**% Increase**
Pro-inflammatory Cytokine panel			
IL 2	8.3±0.18	438.61 ± 0.09	98.17
IL 6	761 ± 0.04	942.84 ± 0.02	19.21
IL 12p70	7.2 ± 0.04	321.12 ± 0.2	97.81
IL 17A	8.8 ± 0.2	317.8 ± 0.18	96.26
IFN α	6.83 ± 0.06	548.07 ± 0.02	98.90
IFN γ	8.49 ± 0.6	216.61 ± 0.78	96.29
TNF α	683.55 ± 0.78	906.25 ± 0.06	24.61
Growth factor panel			
GM CSF	121.68 ± 0.56	728.55 ± 0.02	83.37
Anti-inflammatory Cytokine panel			
IL 4	2.19 ± 0.67	55.43 ± 0.06	96.36
IL 5	5.81 ± 0.06	637.21 ± 0.08	99.21
IL 9	7.16 ± 0.05	468.15 ± 0.05	98.50
IL 10	46.62 ± 0.1	490.27 ± 0.078	90.61

CONCLUSION

Nicotine has been reported to have significant effect on the cytokine production by PBMC's. Such effect of nicotine on cytokine, suggests the dosage of nicotine in tobacco products has to be optimized.

Based on the findings related to role of nicotine in autoimmune diseases and controlling inflammation in COVID-19 patients, addiction and risks associated with commercial formulation should be considered.

REFERENCES

1. Reddy SS, Shaik Hakh. Estimation of nicotine content in popular Indian brands of smoking and chewing tobacco products. *Indian J Dental Res*. 2008; 19(2): 88–91p.
2. Gonzalez-Rubio J, Navarro-Lopez C, Lopez-Najera E, Lopez-Najera A, Jimenez-Diaz L, Navarro-Lopez JD and Najera Al. Cytokine Release Syndrome (CRS) and nicotine in COVID-19 patients: trying to calm the storm. *Front Immunol*. 2020; 11: 1359. DOI: 10.3389/fimmu. 2020. 01359
3. Ranta N, Turpeinen H, Oksanen A, Hämäläinen S, Huttunen R, Uusitalo-Seppälä R, *et al.* The plasma level of proprotein convertase furin in patients with suspected infection in the emergency room: a prospective cohort study. *Scand J Immunol*. 2015; 48(3): 173–177p.
4. Abdel Massih AF, Ye J, Kamel A, Mishriky F, Ismail HA, Ragab HA, *et al.* A multicenter consensus: A role of furin in the endothelial tropism in obese patients with COVID-19 infection. *Obesity medicine*. 2020; 19: 100281. Available at: https://doi.org/10.1016/j.obmed.2020.100281
5. Gomes JP, Watad A, Shoenfeld Y. Nicotine and autoimmunity: the lotus' flower in tobacco. *Pharmacol Res*. 2018; 128: 101–109p. doi: 10.1016/j.phrs.2017.10.005.
6. Mallone R, Mannering SI, Brooks-Worrell BM, Durinovic-Belló I, Cilio CM, Wong FS, *et al.* Isolation and preservation of peripheral blood mononuclear cells for analysis of islet antigen-reactive T cell responses: position statement of the T-Cell Workshop Committee of the Immunology of Diabetes Society. *Clin Exp Immunol*. 2011; 163(1): 33–49p. doi:10.1111/j.1365-2249.2010.04272.x
7. St-Pierre S, Jiang W, Roy P, Champigny C, LeBlanc E, Morley BJ, *et al.* Nicotinic acetylcholine receptors modulate bone marrow-derived pro-inflammatory monocyte production and survival. *PLoS One*. 2016; 11: e0150230.
8. Buszko M, Park JH, Verthelyi D, Sen R, Young HA, Rosenberg AS. The dynamic changes in cytokine responses in COVID-19: a snapshot of the current state of knowledge. *Nat Immunol*. 2020; 21: 1146–1151p. Available at: https://doi.org/10.1038/s41590-020-0779-1
9. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol*. 2020; 20: 355–362p.
10. Chen LD, Zhang ZY, Wei XJ, Cai YQ, Yo WZ, Wang MH, *et al*. Association between cytokine profiles and lung injury in COVID-19 pneumonia. *Respir Res*. 21, 201 (2020). Available at: https://doi.org/10.1186/s12931-020-01465-2.
11. Polosa R, Caci G. COVID-19: counter-intuitive data on smoking prevalence and therapeutic implications for nicotine. *Internal Emerg Med*. 2020; 15(5): 853–856p. Available at: https://doi.org/10.1007/s11739-020-02361-9.
12. Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell L, Chernyak Y, *et al.* Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. *BMJ*. 2020; 369: m1966.

