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Research Article

Comparison of CD4 lymphocyte subset count between smokers and non-smokers

G. Sachidananda [1], **Santosh Kumar S.C [2]***, Smitha. P.S [3], Yogesh Belagali [4] and Avdhoot Kulkarni[2]

[1]*Department of Physiology, Shridevi Institute of Medical Sciences and Research Hospital. Tumkur, India.*
[2]*Department of Pharmacology, Shridevi Institute of Medical Sciences and Research Hospital. Tumkur, India.*
[3]*Department of Physiology, Sapthagiri Institute of Medical Sciences and Research Hospital. Bangalore, India.*
[4] *Department of Pharmacology, Bharati vidyapeeth Medical College, Sangli, Maharashtra. India.*

*** Correspondence Info:**
Dr. Santosh Kumar S.C,
Tutor, Department of Pharmacology,
Shridevi Institute of Medical Sciences and Research Hospital. Tumkur, India.
E-mail: santoshkurugod@gmail.com

Abstract

Cigarette smoking has been associated with imbalance in cytokine production by affecting the efficiency of peripheral blood mononuclear cells to secrete cytokines. Smoking may suppress maturation of dendritic cells in the lymph nodes which ultimately suppress function of CD4+ T cells. The present study was intended to know the association of CD3, CD4 count between Smokers and non smokers. CD3 count of smokers and non smokers is 2593.21 ± 212.09 and 2134.29 ± 145.77. CD4 of smokers and non smokers is 1194.94 ± 50.06 and 1119.16 ± 77.77. In our study we didn't find any statistically significant differences in CD3 and CD4 lymphocyte count between smokers and non-smokers. The other general parameters like blood pressure and chest circumference of smokers and non smokers have also shown no significant difference.

Key words: Cigarette Smoking, CD4, CD3, Smokers, non smokers.

1. Introduction

Cigarette smoking is a worldwide epidemic and the most prevalent cause of many diseases [(1)] which may significantly increases the risk for heart disease, cancers of various organs, and acute and chronic respiratory tract infections [(2)]. Tobacco smoking is characterized by profound immunological changes which impairs host defence and increase susceptibility to infections[(3)]. Smoking related systemic immunomodulation has been previously investigated in terms of humoral and cell mediated immunity [(3)] and it is known that smoking alters the course of diseases such as AIDS[(4)] and influenza[(5)].

Smoking affects host innate immunity including structural and functional changes in respiratory ciliary epithelium, lung surfactant protein, and immune cells such as alveolar macrophages, neutrophils, lymphocytes and natural killer (NK) cells [(6)] and also smokers showed significantly lower NK cell activity than the non-smokers [(7)]. Cigarette smoking has been associated with imbalance in cytokine production[(8)] by affecting the efficiency of peripheral blood mononuclear cells to secrete cytokines[(9)]. Smoking also up regulates the expression of Fas (cell surface molecules mediating apoptotic cell death) on peripheral blood lymphocytes, rendering them susceptible to apoptosis [(10)]. Smoking may suppress maturation of dendritic cells in the lymph nodes which ultimately suppress the function of CD4+ T cells [(11).] Cigarette smoke inhibits the antibody-forming cell (AFC) response, and this immunosuppression is causally related to impairment of antigen-mediated signalling in T cells [(12).] Chronic exposure to cigarette smoke or nicotine depletes inositol-1, 4, 5-trisphosphate (IP3)-sensitive intracellular Ca^{2+} stores, and may account for the immunosuppression and the loss of T cell function[(2)] and a study demonstrated that chronic exposure of rats to nicotine inhibits the antibody-forming cell response, impairs the antigen-mediated signalling in T cells, and induces T cell anergy [(13)]. CD4 count changes with that of CD3 count.

Hence the present study was intended to know the association of CD3, CD4 count between Smokers and non smokers.

2. Materials and Methods

Thirty young men of healthy volunteers of age group 20-30 years who satisfy the inclusion criteria for the study were recruited. Smoking history was obtained by a detailed questionnaire. Informed consent was taken.

2.1 Ethical Clearance: was obtained from Institutional Ethics Committee for human research.

2.2 Inclusion Criteria:

i. Age group of 20-30 years
ii. Smoking history ≥ two years

2.3 Exclusion Criteria:

History of

i. Allergic disorders
ii. Any immunological disorders

iii. Alcoholics
iv. Diabetes
v. Subjects on steroid and anticonvulsive therapy
vi. Passive Smokers

General parameters like blood pressure (systolic and diastolic), chest circumference at the level of 4th intercostal space during inspiration and expiration were measured. Blood pressure was recorded thrice, in the sitting posture, right arm cuffed, for each subject at an interval of 5 minutes and the average of the last two recordings were taken.

CD4 and CD3 analysis was done using flow cytometer Fluorescent activated cell sorter scan (version 3.1), (BECTON DICKINSON MODIFIT LT)

2.4 Procedure followed for CD4 Analysis:

a. 5 ml venous blood sample was collected in K3 EDTA vaccutainers.
b. Trucount tube (Reametrix), which contain anti CD3 and CD4 monoclonal antibody coated to the tube was taken.
c. 20µl of the antibody conjugate was added to the bone of the test tube.
d. 50 µl of whole blood was added
e. Vortex the entire mixture in the trucount tube for 30 sec.
f. Incubated in dark for 15 mins.
g. 450 µl of 1X lysing solution was added (Lyses RBC cells)
h. Again vortex the entire mixture in the trucount tube for 30sec.
i. Again incubated in dark for 10 mins at room temperature.
j. Acquisition of the sample was done in the flow cytometer.
k. CD4 Lymphocytes are calculated from CD3 cells.

3. Results

Table 1: Age Distribution and General Parameters of non smokers and smokers

Age Distribution & General Parameters (Mean ± SD)	Non-smokers	Smokers	P value
Age in Years	25.53 ± 2.83	25.67 ± 2.85	0.856
Blood Pressure in mm Hg (Systolic BP)	110.00 ± 12.03 (90-130)	111.33 ± 11.96 (90-130)	0.132
Blood Pressure in mm Hg (Diastolic BP)	83.00 ± 10.55 (70-110)	79.33 ± 7.85 (70-110)	0.668
Chest circumference (Inspiration)	103.10 ± 10.05	102.93 ± 9.58	0.948
Chest circumference (Expiration)	98.70 ± 9.85	98.20 ± 9.24	0.840

Table 2: Duration of Smoking

Duration in years	Number (30)
≥ 2 years	2
2-4 years	13
4-6 years	12
6-8 years	3

Table 3: Comparison of CD3 and CD4 between non smokers and smokers

Parameters	Non-smokers	Smokers	P value
CD3	2593.21 ± 212.09 (1531.68-8031.47)	2134.29 ± 145.77 (1037.58-4672)	0.084
CD4	1194.94 ± 50.06 (833.99-1650.70)	1119.16 ± 77.77 (433.89-1925.57)	0.410

Table 1 represents the age distribution and the general parameters of the study; comparison of blood pressure and the chest circumference between non smokers and smokers. Samples are age matched with P=0.856. Blood pressure and chest circumference is statistically similar between two groups with P>0.5. {Results are presented in Mean ± SD (Min-Max)}. Table 2 represents the duration of smoking between non-smokers and smokers. Table 3 represents the comparison of CD3 and CD4 count between smokers and smokers.

4. Discussion

In the present study all the general parameters which include systolic BP, chest circumference during inspiration and expiration has showed no significant difference between smokers and non smokers which is in contrast with the other study where they have found an effect of smoking on blood pressure and Chest circumference [14] [15] [16]. These differences probably may be due to differences in ethnicity, duration of smoking in these individuals, age of the subject selected and the percentage of nicotine in the cigarettes they smoked.

CD4 count changes with that of CD3 count; therefore we have considered CD3 count also. Our study didn't find any statistically significant differences in CD3 and CD4 count between smokers and non-smokers. Previous studies identified the effect of cigarette smoking on blood CD4 cell counts, and have concluded that smoking may not have a marked effect on clinical outcome [17] [18] and the other cohort studies didn't find an association between cigarette smoking and HIV disease progression [19] [20] [21]. Hence the previous studies data suggests that the cigarette smoking may not have an association with that of blood CD4 cell counts and HIV disease progression but we cannot completely rule out the effect of cigarette smoking on CD4 cells since smoking may suppress maturation of dendritic cells in the lymph nodes which ultimately suppress the function of CD4+ T cells [11].

5. Limitations of the Study

Since this study was carried out in thirty smokers in the selected population who were smokers of different cigarette brands, sizes. A similar study with a larger sample size may yield better results and may confirm the findings of the study and if the study also incorporates the nicotine percentages of the cigarette.

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