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The Patterns of some Inflammatory Cytokines, Liver Enzymes, and Oxidative Markers Circulating amongst Prolonged Cigarette Smokers

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Abstract

Objective(s): The present study was designed to provide additional information on the levels of cytokine responses, endothelial injury, and oxidative stress responses during prolonged cigarette smoking using the serum levels of interleukin-1β, IL-10, CRP, liver enzymes (aspartate transaminase, alanine transaminase, alkaline phosphates), creatine kinase-MB, troponin T and malondialdehyde (MDA) on prolonged smokers.

Materials and methods: A total of 204 subjects within the ages of 18 and 55 years were randomly studied. Among these were 95 smokers (smoking at least 6 cigarettes per day for more than 6 months) and 109 nonsmoking individuals from Igbinedion University Teaching Hospital Okada Community. Absolute white blood counts were estimated using Sysmex® Automated Hematology Analyzer whereas; IL-1β, IL-10, CRP, MDA, TT, and CK-MB were estimated using enzyme-linked immunosorbent assay methods. Liver enzymes (AST and ALT) were estimated using auto-analyzer.

Results: The levels of IL-10, CRP, MDA, CK-MB, TT, liver enzymes, SBP and DBP were significantly elevated in the smokers as compared with the non-smoking subjects whereas; the levels of IL-1β, AGC and ALC were significantly reduced in the smokers as compared with the non-smoking subjects.

Conclusion: The levels of IL-1β, AGC and ALC were significantly reduced in prolonged cigarette smokers which is indicative of an inhibitory effect on white blood cells. Prolonged cigarette smoking shift of cytokine responses towards IL-10 pattern which might be a result of an imbalance of the Th1 to Th2 type cytokines. The levels of MDA, CRP, CK-MB, TT and liver enzymes (AST and ALT) could be used as an index to assess the degree of lipid peroxidation in prolonged cigarette smoking.

Keywords: Prolonged cigarette smoking; Interluekin-1β; IL-10; Cytokines; Liver enzymes

Introduction

The World Health Organization proposes smoking as a preventable risk factor for endothelial injury such as cardiovascular disease (CVD) [1]. Cigarette smoking is harmful to all organs of the human body and has become the global number two killer after hypertension [2-4]. The possible mechanism by which cigarette smoking may initiate or accelerate endothelial injury might be the activation of free radicals, such as nitric oxide radicals, singlet oxygen, and hydrogen peroxide [3,5]. It is also possible that oxidative stress promotes a systemic acute phase response by activation of nuclear factor kappa B (NF-κB), other cytokines, and adhesion molecules [5]. Several studies have shown the effect of smoking on the serum concentrations of some inflammatory markers, fibrinogen, plasma viscosity, and high-sensitivity C-reactive protein, compared with never smokers, and slightly increased for ex-smokers [3-5].

There is growing body of evidence suggesting the relationship between oxidative stress and cytokine responses. Therefore, in the current study, the cytokine responses, endothelial injury, and oxidative stress responses during prolonged cigarette smoking was investigated using the serum levels of interleukin-1β, IL-10, CRP, liver enzymes (aspartate transaminase, alanine transaminase, alkaline phosphatase), creatine kinase-MB, troponin T and malondialdehyde (MDA) of chronic smokers compared with non-smokers. This will add to the exiting level of information about cigarette smoking as the Federal Ministry of Health in Nigeria has previous warned that cigarette smokers are liable to die young [6]. Major strides towards national tobacco control have been made since Nigeria became signatory to the WHO Framework Convention on Tobacco Control (FCTC) in June 2004 [6]. The Nigeria senate passed a bill in 2011 which when passed into law will be able regulate and control production, manufacture, sale, advertising, promotion and sponsorship of tobacco or tobacco products [6].

Materials and Methods

Subjects

This cross sectional study was conducted at Igbinedion University Teaching Hospital in Okada Community in Ovia North East local Government Area, Edo State, Nigeria. Ethical approval was obtained from IUTH Ethical Review Committee. Informed consent was obtained from all subjects before the commencement of the study. A

total of 204 subjects within the ages of 18 and 55 years were randomly studied. Among these were 95 smokers (smoking at least 6 cigarettes per day for more than 6 months) and 109 nonsmoking individuals from Igbinedion University Teaching Hospital Okada Community. The participants were allowed to rest for at least ten minutes after which the blood pressure and pulse rate were measured from the left arm as described by Musa et al. [7] using an automated digital electronic BP monitor (Omron digital BP monitor, Model 11 EM 403c; Tokyo Japan).

Blood samples analysis

Eight milliliters volume of venous blood sample were collected from the ante-cubital vein of the subjects using standard laboratory collection technique and shared equally into ethylene diamine tetra acetic acid (EDTA) vacutainers and an anticoagulant free vacutainers, subsequently centrifuged at 750 g for 15 min to obtain serum. The blood samples collected in EDTA were used for absolute lymphocyte counts using Sysmex® Automated Hematology Analyzer as previously described by Ehiaghe et al. [8]. The determination of AST and ALT were done using SelectraProS auto-analyzer as described by Mohadesh and Abdolali [9] whereas, the levels of IL-1β and IL-10 were determined using enzyme-linked immunosorbent assay method as described by Khalid et al. [10]. Serum CRP was determined using enzyme-linked immunosorbent assay method as described by Digban and Ehiaghe [11] while the levels of Creatine kinase-MB, Troponin T were determined using enzyme-linked immunosorbent assay methods as described by Ehiaghe et al. [12]. Malondialdehyde estimation was determined using spectrophotometric method as previously described by Ehiaghe et al. [12].

Results

Tables 1 and 2 shows the levels (mean ± SD) of IL-1β, IL-10, CRP, MDA, AGC, ALC, CK-MB, TT, liver enzymes (AST and ALT), SBP and DBP in the smokers as compared with the with the non-smoking subjects. The levels of IL-10, CRP, MDA, CK-MB, TT, liver enzymes (AST and ALT), SBP and DBP were significantly elevated in the smokers as compared with the non-smoking subjects. Whereas, the levels of IL-1β, AGC and ALC were significantly reduced in the smokers as compared with the non-smoking subjects.

Data analysis

Student's t-test was used to compare independent variables. The probability values less than 0.05 were considered significant. The statistical analysis were done using SPSS version 20.0.

Subjects	Interleukin-1β	Interleukin-10	C-reactive protein	MDA	Absolute granulocyte count	Absolute lymphocyte count
Control	1.03 ± 0.04	0.04 ± 0.05	1.20 ± 0.36	1.4 ± 0.04	3.4 ± 0.84	2.3 ± 0.80
Smokers	0.03 ± 0.05	1.23 ± 0.18	9. 3 ± 2.93	3.0 ± 0.04	2.6 ± 0.07	1.3 ± 0.85
P value	0.001*	0.001*	<0.001*	<0.001*	0.035*	0.046*
ns=non significant; *=significant						

Table 1: Levels (mean ± SD) of Interleukin-1β (pg/ml), Interleukin-10 (pg/ml), C-reactive protein (mg/L), MDA (umol/L), Absolute granulocyte count (Cells/µl) and Absolute lymphocyte count (Cells/µl) in prolonged cigarette smokers as compared with the with the non-smoking subjects.

Subjects	Creatine kinase-MB	Troponin-T	Aspartate transaminase	Alanine transaminase	SBP	DBP
Control	40.8 ± 3.8	0.04 ± 0.06	10.4 ± 0.50	9.5 ± 4.11	184 ± 8.50	80 ± 6.3
Smokers	54.0 ± 2.06	27.6 ± 4.5	32.0 ± 0.60	33.5 ± 0.4	194 ± 9.58	87 ± 0.20
P value	0.03*	0.012*	0.03*	0.04*	0.002*	0.001*
ns=Non Significant; *=Significant; SBP=Systolic Blood Pressure; DBP = Diastolic Blood Pressure						

Table 2: Levels (mean ± SD) of Creatine kinase MB (ng/ml), Troponin-T (pg/ml), Aspartate transaminase (U/L), Alanine transaminase (U/L), SBP(mmHG), and DBP (mmHG) in prolonged cigarette smokers as compared with the non-smoking subjects.

Discussion

This is the first study in Nigeria evaluating the serum levels of interleukin-1β, IL-10, CRP, liver enzymes (aspartate transaminase and alanine transaminase), creatine kinase-MB, troponin T, malondialdehyde (MDA), SBP and DBP of prolonged cigarette smokers compared with nonsmokers. The levels of IL-1β, AGC and ALC were significantly reduced in the smokers as compared with the non-smoking subjects. Thus, confirming the inhibitory effect of prolonged cigarette smoking on white blood cells. Also, a very intriguing finding was the shift of cytokine responses towards IL-10 (an anti-inflammatory cytokine) pattern with the suppression of IL-1β which is a pro-inflammatory cytokine. This might be a reflection of a local shift in the Th1 to Th2 type cytokine balance which could suggest that PCS could be an adjuvant of adaptive Th2 immunity. Ouyang et al. [13] reported that cigarette smoke contains potent inhibitors such as catechol and nicotine which can possibly shift the cytokine balance towards the anti-inflammatory patterns. The major components of cigarette smoke that may lead to deleterious effects include nicotine, tar, ammonia, carbon monoxide, carbon dioxide, acetone, nitrogen

oxides and cadmium [14]. Many of these agents are known to be immunosuppressive by affecting both innate and adaptive immune response [15,16]. Also, other studies have observed that the number of interferon gamma secreting cells was extremely reduced in prolonged cigarette smokers [17,18]. T-cell activation, proliferation and expression of the cytotoxic proteins are significantly reduced by exposure to cigarette smoke [19]. Spleen cells from animals that are subjected to the heavy dose of cigarette smoke have a significant reduction in their natural killer cell-mediated lytic activity [20]. Cigarette smoke impairs NK cell-dependent tumor immune surveillance which further suggests that the immune response to antigens is altered in these cells [21]. Thus it appears that cigarette smoke may alter the number and type of lymphocyte population in the lungs, activation and expression of cytokines. This in turn leads to functional defects in adaptive immune responses by these cells predisposing to infections.

Furthermore, the levels of CRP, MDA, CK-MB, TT, liver enzymes (AST and ALT), SBP and DBP were significantly elevated in smokers as compared with the non-smoking subjects. This is indicative an increased oxidative stress during prolonged cigarette smoking. Thus, it could be extrapolated that prolonged cigarette smoking can significantly increases the level of lipid peroxidation in smokers. This may occur due to nitrosative stress which is a condition that occurs when the production of highly reactive nitrogen-containing chemicals such as nitric oxide exceed the ability of the human body to neutralize and eliminate free radicals. Therefore, the levels of MDA in combination with CRP, CK-MB, TT and liver enzymes (AST and ALT) could be used as an index to assess the degree of lipid peroxidation in cigarette smokers. It has been reported that smokers are prone to lipid oxidation from the inhalation of a large number of gas-phase and other radicals which gives rise to increase oxidative stress in the hepatic and cardiac muscles [22-24]. Furthermore, Agarwal [25] opined that prolonged cigarette smoking can induce oxidative stress by stimulating NADPH oxidase and decreasing antioxidant defenses.

Conclusion

The levels of IL-1β, AGC and ALC were significantly reduced in prolonged cigarette smokers which is indicative of an inhibitory effect on white blood cells. Also, a prolonged cigarette smoking shift of cytokine responses towards IL-10 pattern which might be a result of an imbalance of the Th1 to Th2 type cytokines. Furthermore, the levels of MDA, CRP, CK-MB, TT and liver enzymes (AST and ALT) could be used as an index to assess the degree of lipid peroxidation in prolonged cigarette smoking.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

This work was carried out in collaboration between all authors. Authors DAK and EFA designed the study and performed the statistical analysis. Author EFA conducted and managed the laboratory analysis. All authors read and approved the final manuscript.

References

1. Lao XQ, Jiang CQ, Zhang WS, Adab P, Lam TH, et al. (2009) Smoking, smoking cessation and inflammatory markers in older Chinese men: The Guangzhou Biobank Cohort Study. Atherosclerosis 203: 304-310.
2. Chang RC, Ho YS, Wong S, Gentleman SM, Ng HK (2014) Neutropathology of Cigarette Smoking. Acta Neuropathol 127: 53-69.
3. Asthana A, Johnson HM, Piper ME, Fiore MC, Baker TB, et al. (2010) Effects of smoking intensity and cessation on inflammatory markers in a large cohort of active smokers. Am Heart J 160: 458-463.
4. Dwyer-Lindgren L, Mokdad AH, Srebotnjak T, Flaxman AD, Hangen GM, et al. (2014) Cigarette smoking prevalence in US Countries. Popul Health Metro 12: 5-10.
5. Fröhlich M, Sund M, Löwel H, Imhof A, Hoffmeister A, et al. (2003) Independent association of various smoking characteristics with markers of systemic inflammation in men: Results from a representative sample of the general population (MONICA Augsburg Survey 1994/95). Eur Heart J 24: 1365-1372.
6. Agaku I, Akinyele A, Oluwafemi A (2012) Tobacco Control in Nigeria-Policy recommendations. Tobac induced dis 10: 8-10.
7. Musa DL, Ibrahim DM, Toriola AL (2002) Cardiorespiratory fitness. J Hu Mov Stu 42: 455-457.
8. Ehiaghe FA, Onyenekwe CC, Akosile CO, Okoye C, Maruf FA, et al. (2016) The expression pattern and role of interferon gamma genes in exhaustive exercise. J Advan Bio 9: 1903-1910.
9. Mohadeseh M, Abdolali R (2015) Sub-lehal effects of copper oxide nanoparticles on blood parameters. J Bio Env Sci 6: 283-291.
10. Khalid MS, Mohammad A, Yusuf FD (2016) The response of pre-inflammatory cytokine factor to different exercise (endurance, resistance, concurrent) in over weight men. Alex J Med 52: 367-370.
11. Digban K, Ehiaghe FA (2017) The levels of serum C-reactive protein and creatine kinase-MM in human immunodeficiency virus seropositive subjects co-infected with Plasmodium falciparum. Intern J Immun 5: 37-40.
12. Ehiaghe FA, Akinshipe BO, Ehiaghe IJ, Josiah S, Agbonlahor DE, et al. (2017) The levels of C-reactive protein, malondialdehyde and absolute lymphocyte count in pre and post-acute exercise. J Sport Med Dop Stud 7: 1-4.
13. Ouyang Y, Virasch N, Hao P, Aubrey MT, Mukerjee N, et al. (2000) Suppression of human IL-1β, IL-2, IFN-γ, and TNF-α production by cigarette smoke extracts. J Allergy Clin Immunol 106: 280-287.
14. Rennard SI (2004) Cigarette smoke in research. Am J Respir Cell Mol Biol 31: 479-480.
15. Sopori ML, Kozak W (1998) Immunomodulatory effects of cigarette smoke. J Neuroimmunol 83: 148–156.
16. Sopori ML, Kozak W, Savage SM, Geng Y, Kluger MJ (1998) Nicotine induced modulation of T Cell function. Implications for inflammation and infection. Adv Exp Med Biol 437: 279-289.
17. Hagiwara E, Takahashi KI, Okubo T, Ohno S, Ued A, et al. (2001) Cigarette smoking depletes cells spontaneously secreting Th (1) cytokines in the human airway. Cytokine 14: 121-126.
18. Sunyer J, Forastiere F, Pekkanen J, Plana E, Kolz M, et al. (2009) Interaction between smoking and the interleukin-6 gene affects systemic levels of inflammatory biomarkers. Nicotine Tob Res 11: 1347-1353.
19. Glader P, Moller S, Lilja J, Wieslander E, Lofdahl CG, et al. (2006) Cigarette smoke extract modulates respiratory defence mechanisms through effects on T-cells and airway epithelial cells. Respir Med 100: 818-827.
20. Sopori ML, Gairola CC, DeLucia AJ, Bryant LR, Cherian S (1985) Immune responsiveness of monkeys exposed chronically to cigarette smoke. Clin Immunol Immunopathol 36: 338-340.
21. Lu LM, Zavitz CC, Chen B, Kianpour S, Wan Y, et al. (2007) Cigarette smoke impairs NK cell-dependent tumor immune surveillance. J Immunol 178: 936-943.

22. Frei B, Forte TM, Ames BN, Cross CE (1991) Gas Phase Oxidants of Cigarette Smoke induced Lipid Peroxidation and Changes in Lipoprotein properties in Human Blood Plasma. Biochem J 277: 133-138.

23. Pryor W, Stone K (1993) Oxidants in Cigarette Smoke; Radicals, hydrogen peroxide, peroxynitrate and peroxynitrite. Ann NY Acad Sci 686: 12-27.

24. Lykkesfeldt J, Viscovich M, Poulsen H (2003) Ascorbic acid recycling in Human Erythrocytes in induced by Smoking in Vivo. Free Radic Biol Med 35: 1439-1447.

25. Agarwal R (2005) Smoking, Oxidative stress and Inflammation: Impact on resting energy expenditure in diabetic nephropathy. BMC Nephrol 6: 13-21.