ORIGINAL ARTICLE

Effect of Chronic Exposure to Biomass Smoke on Markers of Endothelial Inflammation

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ABSTRACT

It is a well-known fact that indoor exposure to biomass fuel smoke is associated with some sort of cardiovascular disease via lung mediated inflammation, oxidative stress and endothelial inflammation. We sought out to find the association between exposure to biomass smoke and selected markers of endothelial inflammation. We compared total leukocyte count, neutrophil lymphocyte ratio, platelet count, serum hs-CRP and lipid profile in 36 women who have been using biomass fuel vs. 36 women who have been using Liquefied pressure gas (LPG) for at least five years. When compared all the markers of endothelial inflammation like total leukocyte count, neutrophil count, lymphocyte count, neutrophil lymphocyte ratio, serum triglyceride, serum cholesterol and serum hs-CRP were found to be higher in biomass smoke exposed group. Moreover, all markers showed positive correlation except serum HDL which showed negative correlation when correlated with years of cooking with biomass fuel.

Keywords: Biomass fuel, endothelial inflammation, cardiovascular disease

INTRODUCTION

Household air pollution (HAP) from burning of biomass smoke has been recognised as the third leading risk factor for death worldwide, [1] and it is estimated to affect 3 billion people each year. Exposure to biomass smoke is a major cause of illness and death in developing countries.[2] Its exposure has been linked with increased risk of various diseases like acute and chronic pulmonary tract infections in children and adults[3], emphysema, [4] tuberculosis[5] and cardiovascular disease (CVD)[6]

A healthy endothelium is essential for proper functioning of the vasculature. The inflammatory response is a key mechanism in the pathogenesis of atherosclerosis and its progression.[7] Neutrophils secrete inflammatory mediators that can cause vascular wall degeneration. Conversely, lymphocytes regulate the inflammatory response and thus have an anti-atherosclerotic role. Therefore, the neutrophil to lymphocyte ratio (NLR) has been proposed as an inflammatory biomarker[8] and potential predictor of risk and prognosis in CVD. Different lipoproteins have particular effects on vessel health. In the clinical context high levels of LDL and low levels of HDL are particularly important risk factors for CVD and are considered good for vascular health.[9]Highly sensitive-C reactive protein (hs-CRP) also has pro atherogenic effects: it facilitates plaque deposition, transmigration of monocytes and macrophages. [10]Many studies have proposed a relationship between exposure to particulate matter (PM) and initiation of endothelial inflammation leading to an increased risk of cardiovascular events[11].

Specifically, observational studies have found higher blood pressure, a thicker carotid intima–media complex and an increased prevalence of coronary heart disease, stroke and diabetes in populations chronically exposed to biomass fuel smoke. [12]The mechanisms proposed include pulmonary inflammation with release of cytokines in systemic circulation, oxidative stress, endothelial dysfunction and thrombogenesis, all of which could lead to atherosclerosis and adverse health outcomes[13]. Recent research has focused on identifying markers of early endothelial inflammation which could help assess CVD risk earlier in the exposed population.

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MATERIALS AND METHODS

Seventy two females in the age range of 20-40 years were included in this study from Tejgarh Yadgar Shaheedan. It is a small village near Manawa, Lahore. There is no natural gas supply in this village. Poor class use cow dunk cake or grass as a source of energy And affording class use LPG cylinder for cooking and heating purpose. The study protocol was approved by the Ethics committee of the University of Health Sciences, Lahore.

Inclusion Criteria: The inclusion criteria were
(i) Apparently healthy women, (ii) non-smokers, nonconsumption of alcohol and non-chewers of tobacco and (iii) cook regularly with either biomass or LPG at least 2 h/day, 5 days/week for greater than or equal to 10 years. These women were divided into two groups.
Group 1: women who cook exclusively with biomass fuel.
Group 2: women who cook exclusively with LPG fuel.

Exclusion criteria: Mixed fuel user (biomass + LPG + Kerosene), (ii) pregnant, (iii) currently under medication, (iv) family history of Tuberculosis or complicated cardiovascular disease and (v) History of chronic respiratory diseases like asthma, Chronic Obstructive Pulmonary Disease.

After taking informed consent from the subject. Following sampling was done.

Blood Sampling: Six cc venous blood sample was drawn from antecubital vein of each subject. Four ml was added in serum tube i.e. red top vacutainer and other 2ml was added in EDTA added purple coloured top vacutainer. Blood in red vacutainer was centrifuged (1600g for 15 minutes), serum was separated, divided into aliquots and frozen at -80 °C to be used later for analysis.

Complete Blood Count Analysis: For complete blood count, 2cc blood taken in purple top vacutainer (containing ethylene

diamine tetra acetic acid) was used, mixed for 5 minutes on rotator and used for complete blood analysis.

Estimation of lipid profile: Serum cholesterol levels and serum high density lipoprotein (HDL) were estimated by total enzymatic colorimetric method endpoint. Results were read by calorimetric analyser micro lab 300 (USA) at 500 nm Serum triglyceride levels were estimated by the use of enzyme lipoprotein lipase. Serum Low density lipoprotein (LDL) was estimated by the formula Low density lipoprotein (LDL) = Total cholesterol – HDL – (Triglyceride/5).14

Estimation of serum hs-CRP

Serum hs-CRP estimation was done by High Sensitivity C - reactive protein (hs-CRP) ELISA Kit Results were analysed by stipreader, USA.

Data analysis was carried out through computer software IBM SPSS version 21.Quantitative variables were compared by Mann Whitney U test which were non-normally distributed normally distributed were compared by independent t-test as they were normally distributed and presented in the form of mean ± SD. Correlation between years of cooking wth bomass fuel and markers of endothelial inflammation was checked by Spearman's rank correlation. p≤ 0.05 was considered statistically significant.

RESULTS

Median (IQR) of neutrophil count, lymphocyte count, neutrophil lymphocyte ratio NLR and platelet count was higher in group 1 biomass users as compared to LPG users as shown in table-4. When compared by Mann Whitney U test both groups show statistically significant different neutrophil, lymphocyte count, NLR and Platelet count as shown in Table 1.

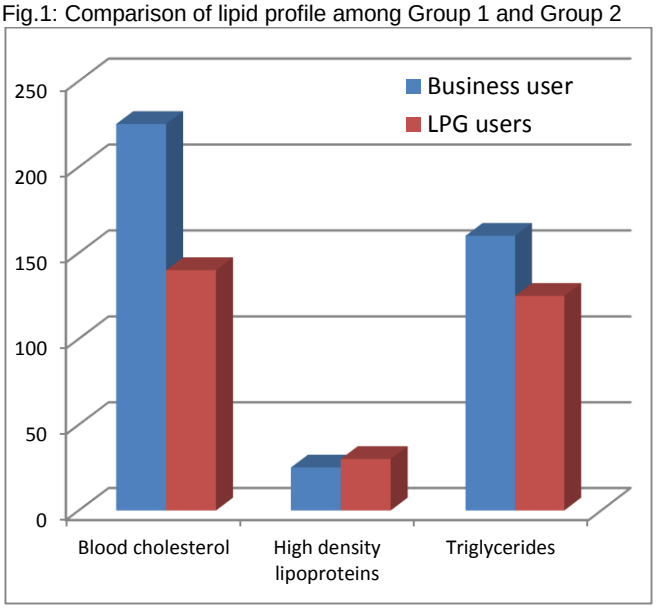
Mean ± SD total leukocyte count of Biomass user was higher than LPG user but didn't show statistically significant difference when compared by independent t-test.

Lipid profile of study population: Table-2 both groups had significant difference in lipid profile. Levels of triglycerides (p-value < 0.001) and Cholesterol (p-value < 0.001) showed significant difference when compared by Mann Whitney U test. Serum LDL (p-value= 0.007) and serum HDL (p-value< 0.001) also showed significant difference when compared by Independent t-test. Levels of serum triglyceride, serum cholesterol, serum LDL were significantly higher n biomass users and serum HDL levels were significantly lower in biomass user when compared with LPG users (Fig.1)

Correlation between years of cooking with biomass fuel and markers of endothelial inflammation: When markers of inflammation were correlated with years of cooking. All markers showed positive correlation except serum HDL which showed negative correlation as shown in table 3.

Comparison of serum hs-CRP in both groups: Levels of hs-CRP (inflammatory marker) was significantly different among two groups as compared by Mann Whitney U test (p-value <0.001).

Fig.1: Comparison of lipid profile among Group 1 and Group 2



*p-value < 0.05 is considered statistically significant.
compared by independent t-test
compared by Mann Whitney U test

Fig.2: Comparison of serum hs-CRP among Group 1 and Group 2

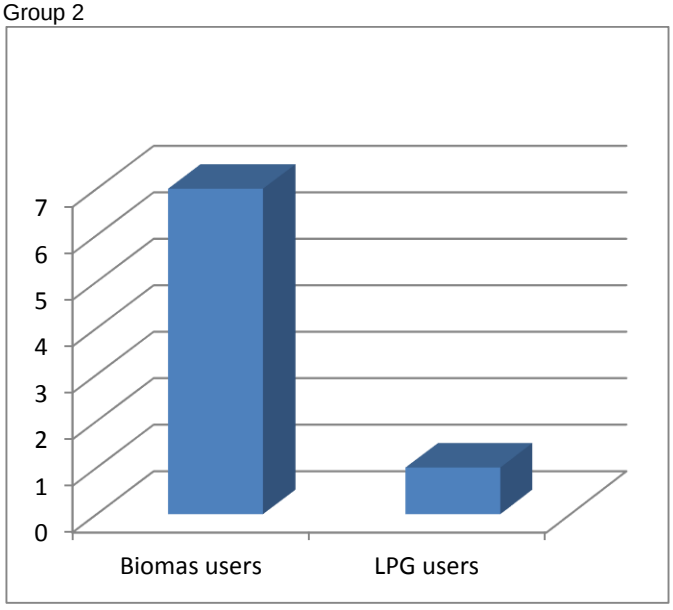


Table 1: Comparison of Hematological Parameters of Group 1 and Group 2.

Hematological Parameters	Group 1 Biomass user (n=36)	Group 2 LPG user(n =36)	P-value
Total leukocyte Count (Mean ± SD)	9.8±2.07	6.1±1.8	0.976
Neutrophil Count Median (IQR)	5.3 (4.9-5.9)	4.60(4.1-4.8)	<0.001*
Lymphocyte Count Median (IQR)	1.90(1.52-2.27)	2.40(2.15-2.50)	<0.001*
Neutrophil Lymphocyte Ratio Median (IQR)	2.87(2.38-3.45)	1.93(1.73-2.07)	<0.001*
Platelet Count Median (IQR)	298.5(272.2-359.5)	211(177.2-243.2)	<0.001*

*p-value < 0.05 is considered significant

Table- 2: Comparison of Lipid profile of group 1 Biomass user and group 2 LPG user

Parameter	Group 1 - Biomass user (n =36)	Group 2-LPG user(n=36)	P-Value
Serum Triglycerides (mg/dl)■	158(135-183)	104 (94.25-132.25)	0.027*
Serum LDL (mg/dl)●	161± 61	124 ± 35	0.007*
Serum HDL (mg/dl)●	35 ± 7	41 ± 6	<0.001*
Serum Cholesterol(mg/dl)■	227.7(179.25-251.5)	130.5(118.2-153.0)	0.036*

Table 3: Spearman Correlation analysis to test an association between years of cooking and cardiovascular risk markers in biomass users and LPG users

Spearman's correlation of years of cooking with endothelial inflammation risk marker	Group 1 (Biomass users)		Group 2 (LPG users)	
	rho value	p-value	rho value	p-value
hs-CRP	0.885*	0.000	-0.593*	0.000
NLR	0.651*	0.000	0.209	0.221
Platelet count	0.655*	0.000	0.270	0.112
Serum cholesterol	0.885*	0.000	0.268	0.115
Serum Triglyceride	0.796*	0.000	-0.476*	0.003
Serum HDL	-0.886*	0.000	0.147	0.394

*correlation is significant at 0.01 level.

DISCUSSION

We measured a select group of serum biomarkers as a proxy for endothelial health. While none of these markers have been proven to be a direct expression of endothelial function, they may provide insight into local inflammation that occurs at the level of endothelial cells (Felmeden and Lip, 2005). In this study, we compared endothelial inflammation biomarkers in participants exposed to biomass fuels and LPG fuel during cooking.

Local inflammation is a strong predictor of endothelial health. The total number of WBCs and each subtype have been reported as predictors of CHD. All blood cells are involved almost in the development and progression of atherosclerosis[14]. Neutrophil count, Lymphocyte count and Neutrophil Lymphocyte ratio shows statistically significant difference ($p < 0.005$) among both groups. Total leukocyte count does not show statistically significant difference among both groups but mean TLC is higher in biomass users as compare to LPG users (9.8±2.7 vs 6.1± 1.8). These results are in accordance with a study conducted in India showing increase TLC in chronic biomass users[15]. Mondal, et al., 2011 has also found increase in neutrophil count, lymphocyte count, eosinophil and other inflammatory cells in women chronically exposed to biomass fuel. [16]Chronic inhalation of particulate matter acting as a foreign body leads to activation of white blood cells. They also favour migration of inflammatory cells from airways to tissue which is depicted by rise in inflammatory cells. Platelet count also show statistically significant difference ($p< 0.005$) among both groups. Ray, 2006 has found increase in platelet activity, lipid platelet aggregates and monocyte platelet aggregates in blood of biomass users[17]. Acute exposure of animals to particulate air pollution leads to activation of platelets and increase thrombogenesis.[18]This rise in platelet count is due to increase inflammatory insult of endothelium which exposes sub endothelium to platelets leading to their activation and adhesion. Increase WBC and platelet count modulate and augment coagulant properties of endothelium suggesting a greater risk for CVD in biomass users.

Components of lipid profile, triglyceride, cholesterol, LDL and HDL shows statistically significant difference ($p<0.005$) among the two study group. According to Framingham heart study rise in levels of these marker is an established risk factor for atherosclerotic cardiovascular disease[19]. Sun, et al., 2005 reported 1.5 fold increase in aortic arch lipid content in mice who are exposed to concentred ambient particles versus filtered air.[20] In a study conducted in Shaxi, China had reported association between dyslipidemia and household solid fuel use[21]. Recent studies have reported that systemic inflammation due to biomass fuel exposure causes dyslipidaemia[22].

Increased hs-CRP is an established risk factor for CVD. Levels of hs-CRP show statistically significant difference ($p<0.001$) among our study population. Same results have been documented by Dutta, et al., 2012 in rural Indian women cooking with biomass fuel. The increased levels of $PM_{2.5}$ in airways and blood leads to increase in levels of acute phase reactant like hs-CRP.[23] However Carvedo et al., 2016 have reported contrary results. They have reported low hs-CRP levels in biomass users[13]. They attributed this finding to the higher physical activity of their participants as they are involved in farming activities and household work as well, whereas women of present study population are involved in household activity only.

When years of cooking are correlated with various cardiovascular risk markers hs-CRP, NLR, platelet count, serum cholesterol and serum triglyceride strong positive correlation (p-value<0.001) is observed whereas serum HDL shows strong negative correlation(p-value$=0.001$). Banarjee et al., 2012 have also found correlation between the biomarkers which causes activation of neutrophils and markers of oxidative stress with years of cooking with biomass fuel. This can be attributed to continuous exposure of particulate matter which increases the inflammatory insult[15].

In short, this study shows that exposure to biomass fuel during daily household cooking increase level of inflammatory markers leading to systemic inflammation reflected by high level of haematological markers (increase NLR, platelet count, TLC) and high hs-CRP in exposed healthy females.

REFERENCES

1. Lim SS, Vos T, Flaxman AD, et al.. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010:

a systematic analysis for the Global Burden of Disease Study 2010.Lancet. 2012; 380:2224–2260.
2. Torres-duque C, Maldonado D, Perez-Padilla R, Ezzati M and Viegi G. Biomass fuels and respiratory diseases: a review of the evidence. Proc. Of the Am. Thor. Soc., 5(5): 577-590.
3. Vinod M. Indoor air pollution from biomass combustion and acute respiratory children in preschool age children In Zimbabwe. Int. J. Epidemiol., 2003; 32(5): 847–853.
4. Rivera RM, Cosio MG, Ghezzo H, Salazar M and Perez-Padilla R. Comparison of lung morphology in COPD secondary to cigarette and biomass smoke. Int. J. of Tuberc. Lung Dis., 2007; 12(8): 972-977.
5. Lakshmi PV, Virdi NK, Thakur JS, Smith KR, Bates MN, Kumar R. Biomass fuel and risk of tuberculosis: a case-control study from Northern India. J. Epidemol. Community Health, 2008; 66(5): 457-461.
6. Miller KA, Siscovick DS, Sheppard L, Shepherd J, Sullivan JH, Anderson GL, Kaufman JD. Long-term exposure to air pollution and incidence of cardiovascular events in women. N. Engl. J. Med., 2007; 356(5): 447–458.
7. Hoffman A, Blum R, Baruch E, Kaplan and Benjamin. “Leukocytes and coronary heart disease,” Atherosclerosis, 2004; 172(1): 1–6.
8. Venkatraghavan L,Tan TP, Mehta J, Arekapudi A, Govindarajulu A, Siu E, Neutrophil Lymphocyte Ratio as a predictor of systemic inflammation - A cross-sectional study in a pre-admission setting, 2015;(4).
9. Yokokawa H, Yasumura S, Tanno K, Ohsawa M, Onoda T, Itai K, et al. Serum low-density lipoprotein to high-density lipoprotein ratio as a predictor of future acute myocardial infarction among men in a. 2.7- year cohort study of a Japanese northern rural population. J Atheroscler Thromb. 2011; 18:89-98.
10. Ji SR, Ma L, Bai CJ, et al. Monomeric C-reactive protein activates endothelial cells via interaction with lipid raft microdomains. FASEB J 2009; 23:1806–16.
11. Helbing T, Olivier C, Bode C, Moser M, Diehl P, et al., Role of microparticles in endothelial dysfunction and arterial hypertension, World J. Cardiol., 2014; 6:1135–1139.
12. Painschab MS, Davila-Roman VG, Gilman RH, Chronic exposure to biomass fuel is associated with increased carotid artery intima-media thickness and a higher prevalence of atherosclerotic plaques. Heart, 2014; 99(14):984–991.
13. Caravedo MA, Herrera PM, Mongilardo N, Ferrari A, Victor G, Davila-Roman Gilman RH, Wise RA, Miele CH, Miranda JJ, Checkley W, et al., Chronic exposure to biomass fuel smoke and markers of endothelial inflammation. Indoor Air., 2016; 26(5): 768–775.
14. Mohammad M, Omid F, Components of the Complete Blood Count as Risk Predictors for Coronary Heart Disease In-Depth Review and Update Tex Heart Inst J. 2013; 40(1): 17–29.
15. Banerjee A, Mondal NK, Das D, Ray, MR, Neutrophilic Inflammatory Response and Oxidative Stress in Premenopausal Women Chronically Exposed to Indoor Air Pollution from Biomass Burning. Inflammation, 2012; 35(2): 671-683.
16. Mondal NK, Bhaumik R, Das CR. Assessment of indoor pollutants generated from bio and synthetic fuels in selected villages of Burdwan, West Bengal. J. Environ. Biol., 2013; 34: 963–966.
17. Ray MR, Mukherjee S, Roychoudhury S, Banarjee M, Siddique S, Chakraborty S, Lahiri T. Platelet activation, upregulation of CD11b/CD18 expression on leukocytes and increase in circulating leukocyte-platelet aggregates in Indian women chronically exposed to biomass smoke. Hum Exp Toxicol., 2006; 25(11): 627-635.
18. Vermylen J, Hoylaerts, HF. The procoagulant effects of air pollution. J Thromb Haemost., 2007; 5(2): 250- 251.
19. Chuang KJ, Yan YH, Chiu SY, Cheng TJ. Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. J Occup Environ Med., 2011; 68(1): 64–68.
20. Sun, Q. Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA., 2005; 294(23): 3003–3010.
21. Weihu QU, Yan Z, Guohua QU, Ikram. Household solid fuel use and cardiovascular disease in rural area in Shaxi, China. Iran J. Public Health, 2014; 44(5): 625-638.
22. Yue W, Schneider A, Stölzel M, Rückerl R, Cyrys J, Pan X, Zareba W, Koenig W, Wichmann HE, Peters A. et al., Ambient source-specific particles are associated with prolonged repolarization and increased levels of inflammation in male coronary artery disease patients. Mutat. Res., 2007; 621(1-2):50-60.
23. Dutta A, Ray MR, Banarjee AR. Systemic inflammatory changes and increased oxidative stress in rural Indian women cooking with biomass fuels. Toxicol. Appl. Pharm., 2012; 261(3):255-262.