Original Article

Increased Risk of Cardiovascular Disease in Women Exposed to Biomass Fuel During Cooking

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Objective: To determine and compare cardiovascular risk markers among women using biomass fuel and those using Low Pressure Gas (LPG) for cooking purpose.

Methods: Seventy two female subjects were recruited for this study. Thirty six women in group 1(using biomass fuel) and 36 in group 2(using LPG fuel) for cooking purpose. After taking informed consent, blood pressure was measured. Air sampling was done at kitchen of both groups for estimation of PM2.5 concentration. Blood sample was taken for estimation of Ischemia modified albumin (IMA), lipid profile and highly sensitive C - reactive protein (hs-CRP). Data was analysed by IBM SPSS version 23.p value < 0.05 was considered statistically significant.

Results: Biomass fuel users have two to four time higher PM2.5 concentration in their kitchen (p-value <0.005). These women have significantly higher Systolic and Diastolic blood pressure (p-value < 0.005). Significantly higher levels of IMA, hs-CRP, Low Density Lipoprotein, Cholesterol, Triglyceride level and lower High Density Lipoprotein (p-value <0.001) level were found in biomass users when compared with LPG using women.

Conclusions: Biomass fuel exposure induces oxidative stress and systemic inflammatory disease thereby increasing the risk of development of atherosclerosis and CVD in exposed persons.

Keywords: biomass fuel, cardiovascular disease, particulate matter 2.5.

Introduction

Pakistani population has one of the highest risks of coronary heart disease (CHD) in the world. CHD deaths in Pakistan has reached about 200,000 per year.[1] During past years, attention was drawn to Indoor Air Pollution (IAP) and its likely role as a risk factor for cardiovascular disease.[2] One of the source of indoor air pollution is burning of biomass fuel which comprises of plant and animal material in the form of wood, charcoal, animal dunk cakes and crop residues. Biomass fuel when burnt emits smoke which contains coarse, fine, and ultrafine particles, transition metals, aldehyde, benzene, fluorine and arsenic (in case of coal burning), volatile organic compounds and bio-aerosols All of these substances have health damaging properties.[3] Majority of rural and semi-urban house-holds in Pakistan have to use biomass fuel against their will due to lack of better alternatives. According to World Health Organization (WHO) estimates, 70,700 deaths in Pakistan were associated with biomass fuel exposure and national burden of disease attributed to biomass fuel use was 4.6%.[4] In 2009, Colbeck and colleagues recorded mass particulate matter concentration in the range of 4000-8555 $\mu g/m^3$ in kitchen during cooking hours.[5] these concentrations are manifold folds higher than the air quality guidelines proposed by WHO. According to WHO, 2010 the 24 hour $PM_{2.5}$ concentration should not rise higher than 25 $\mu g/m^3$.[3,4]

Specifically, observational studies have found higher blood pressure, a thicker carotid intimamedia complex and an increased prevalence of coronary heart disease, stroke and diabetes in populations chronically exposed to biomass fuel smoke.[6] The mechanisms proposed include pulmonry inflammation with release of cytokines in systemic circulation,[7] oxidative stress, [10]endothelial dysfunction[8] and thrombogenesis,[9] all of which could lead to atherosclerosis and adverse health outcomes.[8]

Pakistan offers a good opportunity to study the risk of cardiovascular diseases in relation to indoor air pollution from biomass fuel as the prevalence of cardiovascular disease is 26.9% in Pakistan with prevalence being more in women (30%) as compared to men (23.7%).[1] Sixty six percent of Pakistani population belongs to rural areas and 94% of rural population and 60% of urban population depends on biomass fuel for cooking purposes.[11] There is also limited data evidence from Pakistan on biomass fuel as risk of cardiovascular disease.[12]More people of Pakistan are exposed to biomass fuel because of non-awareness of pros and cons of this fuel, less developed infrastructure of natural gas distribution, insufficient natural gas supply leading to gas load

shedding and cost effectiveness of this fuel for the labour class. Moreover, new guidelines on Household Air Pollution by WHO provides the scientific rationale to conduct this study.

Methods

Seventy two females in the age range of 20-40 years were included in this study from Tejgarh yadgar shaheeda. 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.

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. 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. Subject were asked to sit down. After 5 minutes, their blood pressure was measured from brachial artery by mercury sphygmo- manometer. Three times blood pressure was checked than average was taken. Subjects were than classified into categories according to National Heart, Lung, and Blood Institute, 2003.[13]

Space and Upper Atmosphere Reasearch Comission (SUPARCO), Lahore was requested for air sampling at sampling site. It was done using Thermo Particulate Monitor device model (FH62C14). It is a standard ambient air particulate matter monitoring device which is United State Environmental Protection Agency (USEPA) equivalent designated as shown in Fig 1. Air sampling was done at kitchen of biomass user and LPG user during day time for 4.5 hours while they were cooking meals (taking readings at intervals of 30 minutes).

(Fig.-1) Four ml venous blood sample was drawn from antecubital vein of each subject and dded in serum tube i.e. red top vacutainer Blood in red vacutainer was centrifuged (1600rpm for 15 minutes), serum was separated, divided into aliquots and frozen at -80 °C to be used later for 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] Serum hs-CRP estimation was done by High Sensitivity C - reactive protein (hs-CRP) ELISA Kit Results were analysed by stipreader, USA.

Estimation of serum ischemia modified albumin (IMA). Serum levels of IMA was measured by a calorimetric assay.[15] Data analysis was carried out through computer software IBM SPSS version 21.Quantitative variables like PM2.5 concentration, Blood Pressure, serum triglyceride, serum cholesterol and serum hs-CRP were compared by Mann Whitney U test as they were non-normally distributed and presented in the form of median Intra Quartile Range. Serum IMA, Serum LDL and Serum HDL were compared by independent t-test as they were normally distributed and presented in the form of mean ± SD.

$p \leq 0.05$ was considered statistically significant.

Results

It is apparent that two groups were well matched except for education, family income, physical activity and life style (as more of LPG users do household and field work as well), separate kitchen and kitchen eave space(chimney space). Biomass users were found to be less educated, had lower family income and separate kitchen is found in only 10% of biomass

using household as compared to LPG users.

PM2.5 levels were checked in kitchens of group 1 (biomass user) and group 2 (LPG user) which showed statistically significant difference when compared by Mann Whitney U test (p-value=0.011). Maximum PM2.5 level in kitchen of group 1 (biomass user) kitchen is 472 µg/m3 whereas it was 172 µg/m3 in group 2 (LPG user) as shown in **Fig 2.**

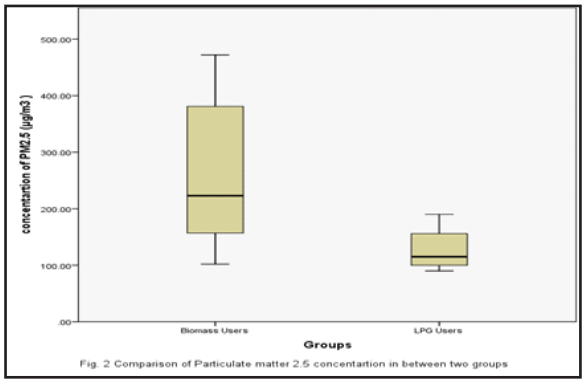

Fig-2: Comparison of particulate matter 2.5 concentration in between two groups.

Table-1: Frequency distribution and comparison of Blood pressure of the study population compared by the study population.

Parameter	Group-2 Biomass user n=36	Group-2 LPG s user n=36	P-value
Blood pressure mmHg	Frequency (%)	Frequency	
Normal (SBP< 120 and DBP<80)	12 (33.3%)	22 (59.7%)	0.029*
Bre-hypertension (SBP 120-139 and DBP<80)	18 (50%)	12 (32.8%)	
Hypertension (SBP ≥140 mmHg and DBP ≥ 90)	6 (16.6%)	2 (6.9%)	

**p-value < 0.05 is considered statistically significant.*

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

Parameter	Group-2 Biomass user n=36	Group-2 LPG s user n=36	P-value
Serum Triqlycerides (mg/dl)	158 (135-183)	104 (94.25-132.25	0.027*
Serum LDL (mg/dl)?	161±61	124±35	0.020*
Serum LDL (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*

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

Table-3: Comparison of hs-CRP and IMA level of the study population by independent t-test and Mann Whitney U test.

Parameter	Group-2 Biomass user n=36	Group-2 LPG s user n=36	P-value
Hs-CRP	7.51 (5.74-8.62)	1.0 (0.6-1.82)	0.001*
Ischemia modified albumin? Mean±SD	0.39±0.12	0.27±0.1	0.001*

**p-value < 0.005 is considered statistically significant*
- *compared by independent t-test*
- *compared by Mann Whitney U test*

Comparison of Blood Pressure of the study population. Both groups showed statistically significant difference when their physical characteristics like systolic blood pressure (SBP) (p-value=0.029), diastolic blood pressure (DBP) (p-value=0.006) were compared by Mann Whitney U test. Median systolic blood pressure, diastolic blood pressure was higher in biomass users when compared with LPG users as shown in table 3. On examination it was found out that 6 biomass users had hypertension whereas only 2 LPG users were found to be hypertensive as shown in **Table-1.**

Discussion

Cardiovascular disease is one of the major cause of death globally. Many factors have been discovered which increase the risk of CVD and exposure to biomass fuel is one of these. Data on exposure to biomass fuel as a cause of CVD is minimal. This study has been done to find out that whether premenopausal women who cook solely with biomass fuel had greater risk of developing CVD than their neighbouring women who cook with LPG fuel.

Many epidemiological studies have found increased risk of cardiovascular disease in people who are exposed to ambient particulate matter daily.[16] $PM_{2.5}$ level in kitchen of both groups shows statistically significant difference (p-value <0.005).In the present study, median $PM_{2.5}$ concentration of eight hour is 223µg/m^3 in biomass using kitchen whereas in LPG using kitchen it is 117µg/m^3. In a study conducted in US had reported that mean $PM_{2.5}$ concentration is 13µg/m^3 [17] whereas average $PM_{2.5}$ concentration in Beijing, China is 112-416µg/m^3 and $PM_{2.5}$ concentration in an Indian village was 156±63µg/m^3.[18] $PM_{2.5}$ concentration in current study is very high in comparison to these studies, which is suggestive of a much higher risk of CVD. Physical characteristics of biomass user kitchen and household could be culprit of this high particulate matter. Most of them have large family size but less number of rooms and no separate room for kitchen and no proper eave space. LPG user kitchen and household characteristics show statistically significant difference (p < 0.005) from them. LPG users have smaller family size, more number of rooms and a separate room for kitchen with a proper eave space in most of the kitchens. Siddiqui et al., 2009 conducted a study (in the area of Rehri Goth, Karachi) and reported that biomass using households had low socioeconomic status with less number of rooms per person per house.[19] Hypertension is considered as a primary risk factor

for CVD.[20] Systolic and diastolic
Blood pressure shows statistically significant difference among groups ($p< 0.001$). Raised blood pressure has been reported in women chronically exposed to biomass fuel in a Guatemalan study[21] and in an Indian study.[18] Particulate matter exposure leads to activation of sympathetic nervous system which plays an important role in development of hypertension. Fine Particulate Matter increases bioavailability of nitric oxide within pulmonary and systemic vascular endothelium. They also induce systemic oxidative stress and increase concentration and activity of vasoconstrictive factors. All these changes are pathogenic of atherosclerosis and hypertension.[22] 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.[23] 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.[24] In 2002, Suwa demonstrated that exposure to particulate matter PM_{10} augmented the total amount of lipids in aortic lesions in animals who were having hyperlipidemia.[25] In a study conducted in Shaxi, China had reported association between dyslipidemia and household solid fuel use.[26] Recent studies have reported that systemic inflammation due to biomass fuel exposure causes dyslipidaemia.[27] 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.[28] However Carvedo et al., 2016 have reported contrary results. They have reported low hs-CRP levels in biomass users.[9] 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.
Oxidative stress is a hallmark of CVD. According to Piva et al., 2011 IMA appears to play the role of an oxidative stress biomarker.[29] Levels of IMA are significantly raised in biomass fuel users as compared to LPG users (p-value <0.005). Rise in IMA level in biomass using women is due to the exposure to biomass smoke which initiates inflammation in respiratory as well as systemic vasculature[30] that results in generation of reactive oxygen species indicating oxidative stress.[20] D'Amoto et al., 2010 also stated that like biomass smoke environmental tobacco smoke also initiates oxidative stress.[31] Also due to low socioeconomic status their diet lacks enough antioxidants which adds to the burden of oxidative stress in these women
In short, this study shows that exposure to particulate matter during daily household cooking increase level of inflammatory markers leading to systemic inflammation reflected by high hs-CRP in exposed healthy females. It also initiates systemic oxidative stress as depicted by high IMA in biomass exposed females. This sustained inflammatory insult and oxidative stress may manifest as increased risk of CVD in biomass exposed females.

Conclusion

Pakistani women who cook with biomass fuel have higher risk of developing cardiovascular disease as concentration of $PM_{2.5}$ is higher in biomass using kitchen. Higher $PM_{2.5}$ level initiate systemic inflammation depicted by increased level of hs-CRP in biomass exposed women. It induces oxidative stress as shown by increased level of IMA in exposed women.
Systemic atherosclerosis indicated by higher blood pressure and deranged lipid profile thus increasing the risk of cardiovascular disease.

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