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Thrombotic and Inflammatory Tendency to Particulate Matter of Woodsmoke

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ABSTRACT

Background: Wood is oldest of human fuels,it is literally true that exposure to woodsmoke is as old as humanity itself. Woodsmoke being a natural substance, it belived that it was considerd benign to human. The elevated level of particulate matter. Would be associated with increased blood levels of thrombotic and inflammatory markers especially in elderly individuals.

The aim of this study was to examine the effects of subchronic exposure to woodsmoke and biomass pollutants on blood coagulation factors, platelets activation and inflammatory markers in the farmers who live in rural areas and used to burn the wood, agricultural products and biomass as an alternative method for domestic heating and cooking.

Subjects and Methods: The study was carried out in the period between Jan. (2007) to Feb. (2008) on 51 exposed subjects to woodsmoke and 40 non exposed subjects as a control. The markers of thrombosis and inflammation were measured (Prothrombin time, activated partial thromboplastin time, thrombin time, fibrinogen levels, coagulation factor VII,VIII, IX D-dimer, von Willibrand factor, CRP, platelets aggregation by ADP and collagen were measured.

Exclusion criteria of the selected groups including: DM, liver and renal disease, Pregnancy, contraceptive bills and smoking, previous history of thrombosis and also family history.

Results: The exposure to particulate matter P.M. of woodsmoke affects blood picture components that leads to a significant increased in absolute neutrophilic count, increase in plateletes count and decrease in closure time of whole blood platelets activation by ADP and collagen ($p<0.05$). As regard to age and sex the results were a significantly increased in WBCs & neutrophiles count in exposed young <30 ys old groups of both sex in comparison to non exposed groups ($p<0.05$).

A significant decreased in closure time of whole blood platelet activation by collagen/ADP only when the exposed (young & old, <30 ys. & >30 ys. old) of both sex groups were compared to non exposed groups of the same age & sex ($p<0.05$). Coagulation profile: the following results were observed: only a.P.T.T clotting times showed a significant decreased when the exposed groups were compared to the non exposed groups ($p<0.05$). FVIII, FIX, & FVIII/vWF ratio showed a significant increase when the exposed groups were compared to non exposed groups ($p<0.05$). F VII & vWF showed a highly significant increase ($p<0.001$). As regard age & sex F VII: a significant increased when the exposed groups of both sex <30 ys & >30ys old) were compared to non exposed groups of the same age & sex ($p<0.05$). Inflammatory markers, Plasma levels of fibrinogen & D-dimer showed a highly significant increase when the exposed groups were compared to the non exposed groups ($p<0.001$), CRP represents a significant increased only ($p<0.05$). As regard age & sex: Plasma level of fibrinogen showed a significant increased when the exposed groups of both sex <30 ys old were compared to non exposed groups of the same age & sex ($p<0.05$). D-dimer level showed a highly significant increased when the exposed young male & female groups were compared to non exposed groups of the same age & sex ($p<0.001$). CRP like D-dimer.

Conclusion: Subchronic exposure to P.M. of woodsmoke and other biomass feul is considerd as one of the risk factors for thrombosis it leads to increased WBCs count, platelets count & activities, so formation what is called platelets-leukocytes aggregates & subsequent thrombosis also it increases the coagulation factors concentration F VIII, vWF, & FVII and plasma level of fibrinogen level and D-dimer. In males >30 ys old, leads to increase of factor VII concentration which is considerd as a risk factor for coronary artery disease.

Key Words: *Woodsmoke – Thrombosis – Inflammation.*

INTRODUCTION

Woodsmoke is a significant source of air pollution in many parts of the world specially in developing countries. Woodsmoke is generated using wood stoves, agricultural ash, in and outside the door **[1]**. Woodsmoke is a major ingredient tiny particles of soot and liquid pollution, it contains carbon monoxide and cancer –causing chemicals **[2]**.

The epidemiological data suggest a causal relationship between elevated wood smoke level and health effects **[3]**. The proposed mechanisms underlying this increase include effects on coagulation factors and inflammatory response **[4]**, increased blood levels of inflammatory cytokines and thrombotic markers specially in eldery. Also increase the oxidative stress **[5]**. P.M. exposure affect blood coagulation as they increase the plasma level of fibrinogen, homocysteine and high sensitive C-reactive protein **[6,7]**. Fine and ultrafine particles affect coagulation cascade, platelete function, leads to atherosclerosis and thrombosis **[8]**. On exposure to biomass smoke, platelet activation, aggregation and formation of platelet-leukocyte aggregates with thrombosis **[9]**.

The rising fossil energy costs has led to increase in the use of wood and other biomass fuels. While the commercial sources of wood combustion have been subjected to some regulation, there are still important unregulated sources woodsmoke including household heating stoves and fireplaces **[10]**.

Two of the principal gaseous pollutants in woodsmoke, CO and NOx, add to the atmospheric levels, haematological alteration occurred due to CO as formation of non functioning haemoglobin e.g. carboxyhaemoglobin **[11]**, why woodsmoke may be a special case requiring separate health evalution **[5]**. Wood consists primarily of two polymers: cellulose (50-70% Wt) and lignin(30% Wt), other biomass fuels also contain these polymersbut in different proportion **[10]**. Woodsmoke particles are generally smaller than 1μm, the range is (0.15-0.4 μm). Fresh woodsmoke contains a large number of ultrafine particles, less than 100μm which condense rapidly as they cool and age. These paticles evade the mucociliary system in the peripheral airway and exert pathogenic inflammatory response. About 5-20% of woodsmoke particulate mass consists of elemental carbon, the composition of organic carbon fraction differe according to fuel being burned and combustion conditions. Woodsmoke is also mutagenic and possibly carcinogenic, but less than coal smoke. A significant woodsmoke exposure, mostly in winter occur indoor and outdoor in all areas of developed world for residential heating **[12]**.

SUBJECTS AND METHODS

Population and study design: The study was carried out in the period between Jan. (2007) to Feb. (2008) on 51 farmers, whom were subjected to subchronic daily exposure to high concentration of woodsmoke (16 males and 35 females) their age ranged from 20 to 45 years old and 40 non exposed subjects as a control (14 males and 26 females), their age range from 20-49 years old. The exposed farmers were selected from the raural areas where burning agricultural biomass, ash and wood were used for indoor and outdoor ordinary human activities e.g. cooking, making bread, frying meat and heating in winter. The studied groups were subclassified according to the age and sex into young groups, whom age below 30 years and more old groups, whom age above 30 years, the exposure in the young groups were more than the old one, as the dueties and acivities in this age group were increase, also in raural areas most of the females above 30 are sitlle dawn, the exposure toparticulate matter P.M is less. The markers of thrombosis and inflammation were measured maximum after 12 hours of exposure to P.M. of woodsmoke. Evaluation the degree of thrombotic tendency and the rise in inflammatory markers by measuring (prothrombin time PT, activated partialthromboplastin aPTT, thrombin time TT, Fibrinogen Level, coagulation factors: FVII, FVIII FIX were done by clotting method on SYSEMEX DADE BEHRING system, vWF was done by latex agglutination method, the kit was supplied by DEAD BEHRING. Platelet activation by ADP/collagen & EPI/collagen was done by whole blood clotting method using PF-100 DADE BEHRING system. CRP was done by latex agglutination method, the kit was supplied by OMEGA. Complete blood count was done on H-MAX Coulter system. Liver and renal function were done on Beckman Synchron CX-9.

Exclusion criteria: Of the selected groups including: DM, liver and renal disease, family and previous history of thrombosis, pregnancy, contraceptive bills and smoking to exclude other risk factors for thrombosis tendency rather than exposure to P.M. of wood and biomass during normal human activities.

Subjects recruitment: Subjects were recruited from the near by villages through advertising them by the mean of the hospital lab. Technich-

iums whom living in nearby villages, also from medical outpatients clinic lab. Medical history and clinical examination were done to exclude cardiac, renal and hepatic disease.

Exposre measures: Atmosphere concentration of particulate matter (P.M.) due to wood burning was measured by the mean of (sequential air sampler, air trap for aerodynamic measurments) in the environmental sanitation center in sohag locality, the size was 2.5μm for fine P.M. and 2.5-10μm for coarse P.M, the conc. Was 265 mμg/m^3.

Blood measures:

About 10ml of venous blood was drawn from every subject within the frist 12 hour after P.M. exposure, 1.8ml was added to each separate citrated tubes (containing 0.2 sod. citrate, one for the coagulation profile and the other tube for the platelet activation). 3ml was drawn to K-EDTA tube for complete blood count performance. The remaining blood was deliverd to plain tube for chemical tests (the vacutainers were supplied by B.D.).

• For coagulation profile the citrated tubes samples were centrifuged in cooling centrifuge system at 4000 rpm for 10 min, at 20ºC to prepare P.P.P., then the poor plasma was deliverd to Sysmex Dead Behring fully automated autoanalyzer system for PT, aPTT, TT, Fibrinogen, coagulaion factors: VII, VIII, IX by clotting method and D-dimer by turbidimetric method. kits supplied by Dead Behring.

• Normal values for each: PT: 10.5-13 sec. & 130-70%, aPTT: 26-38 sec., TT: 14-17 sec, Fibrinogen 180-360 mg/L, coagulation factors: 70%-150%, D-dimer < 20ug/dl.

• For platelet activation the second citrated tube was deliverd to PFA-100 DEAD BEHRING as whole blood activation, using cartilage for ADP and collagen. The closure time was measured. The nomal closure time is for ADP/collagen & forEPI/collagen up to 175 sec.

• vWF was assyed by latex agglutination method supplied by Dead Behring.

• K-EDTA blood tube was subjected to H-MAX fully automated Coulter system for C.B.C.

• The remaining blood was left for clotting, centrifuged, the serum was delivered into Beckman Synchron CX-9 fully automated chemical analyzer for glucose, renal and liver functions. The remaining serum was subjected to CRP by latex agglutination method supplied by OMEGA.

Statistical analysis:

SPSS program was used for data analysis. *t*-test and two way ANOVA test were used (test of significant: *p*-value <0.0.

RESULTS

Environmental measures:

The mean concentration (±S.D) for coarse P.M in the burning field is 16.4±5μg/m^3, the mean concentration for fine P.M was 5±3μg/m^3 in the burning field. The data obtained from the Sohag Sanitation Center.

Time lag after exposure is 12 hours at least.

Subjects: 51 farmers, whom were subjected to subchronic daily exposure to high concentration of woodsmoke (16 males and 35 females) their age ranged from 20 to 45 years old with the mean age (31.6±7.4 SD) and 40 non exposed subjects as a control (14 males and 26 females), their age range from 20-49 years with the mean age (35.5±6.8 S.D). Age and sex of the studied groups were illustrated in Table (1).

Changes in blood picture: Neutrophilia was observed in 8 subjects (15.6%) of exposed group in comparison to 3 subjects (7.5%) of non exposed control group, the mean neutrophiles count was (4.8±2.4S.D) in exposed group versus (4.3±1.4 S.D) in non exposed control group (*p*-value <0.02). Absolute monocytes count was non significantly increased in exposed group versus control group (*p*-value >0.1). Absolute lymphocytes showed a non significant decrease in exposed group versus control group (*p*-value >0.05). Eosinophile and basophile counts were not affected in comparison of both groups (*p*-values >0.1 & >0.2 for both). When the blood picture variables were analysed as regard to age and sex, the young male and female groups <30 years old showed a significant increase in total WBCs and neutrophile counts in exposed groups versus non exposed groups (*p*-value <0.05, <0.005). Hb concentration and HT were significantly increased in exposed males (>30 ys & <30 ys) versus non exposed female groups of th same age (*p*-value 0.02). Reticulocytes count showed a significant increase in exposed

young female group versus control non exposed young one (p-value <0.05). Tables (2,3), Fig. (1) demonstrate the C.B.C changes.

Blood platelets changes: Blood platelet count was significantly increased in the exposed group versus non exposed control group (p<0.05), MPV and Pct showed a non significant variation (p>0.05). Platelet activation by ADP/collagen and EPI/collagen showed a significant decrease in closure time in exposed group in comparison to non exposed group (p-value <0.05). When analysed as regard to age and sex, platelet count showed a non significant variation (p>0.05), MPV represents a significant increase in exposed young male and female groups when compared to non exposed young male and female groups (p<0.05). Platelet crite and PDW showed no significant variation as regard to age and sex (p>0.05). Whole blood platelet activation closure time by ADP/collagen were significantly decreased in exposed young male and female <30 ys when compaired to the same age & sex groups (p<0.05), also a significant decreasewas found in closure time of platelet activation by ADP/collagen in exposedyoung female below 30 years old in comparison to exposed young male group (p<0.05). Platelet activation closure time by EPI/collagen when analysed as regard to age and sex only a significant decrease was found when the exposed old female group more than 30 years was compared to non exposed old female group (p<0.05). Blood platelet changes were listed in Tables (4,5), Figs. (2,3).

Table (1): Age & sex of the studied groups.

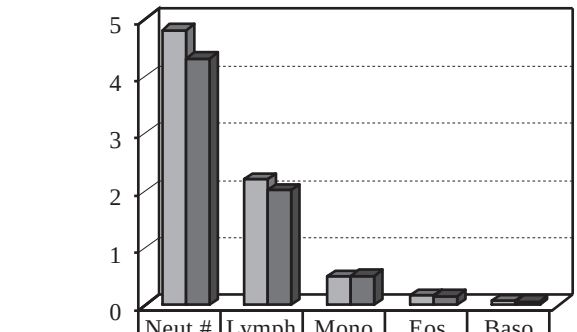
	Exposed group	Non exposed group
No. of subjects	51	40
Age: range	20-45	20-49
Mean±S.D	31.6±7.4	35.5±6.8
Sex	M:16 F:35	M:14 F:26
No. of subjects <30 Ys	M:6	M:6
age: (range)	(20-29 Ys)	(23-28Ys)
	24.2±5.3	25.7±2.6
mean±S.D	F:15	F:12
	(20-28 Ys)	(20-29Ys)
	23.5±4.4	26.7±2.8
No. of subjects >30 Ys	M:10	M:8
age: (range)	(30-45Ys)	(35-46Ys)
	38±5.5	41.2±5
Mean±S.D	F:20	F:14
	(32-45 Ys)	(31-49 Ys)
	37.7±6.8	38.5±5.7

M: Male. F: Female.

Table (2): The blood picture variables in studied groups.

Varibles	Exposed group range Mean±SD	Non exposed group range Mean±SD	p values
WBCs x 10^9/L	3.3-14 7.7±2.4	3.3-11 6.8±2.02	>0.07 NS
RBCs x 10^{12}/L	3.6-5.5 4.4±0.4	4.2-5.5 4.77±0.4	>0.06 NS
Hb g/dl	11-15.4 12.8±1	11.5-15.4 13.3±0.85	>0.06 NS
MCV fl	73-94 73.9±5.6	74.4-94 81.9±5.03	>0.07 NS
Ht%	31.4-47 38.4±3.2	34.9-45 39.2±2.65	>0.08 NS
MCH pg	25.8-34 28.7±2	26-34.4 28.4±1.9	>0.8 NS
MCHC g/dl	32-38.6 35±1.3	33.2-37.5 35.2±1.05	>0.9 NS
RDW%	11-17.4 13.±1.7	11-16 12.9±1.04	>0.06 NS
Neutrophiles# x10^9/l	1.3-10 4.8±2.1	1.7-7.9 4.3±1.43	<0. 02 S*
Lymphocytes # x10^9/l	0.66-4.5 2.18±0.8	0.8-3.5 2±0.71	>0.08 NS
Monocytes# x10^9/l	0.2-0.9 0.5±0.17	0.2-0.9 0.51±0.16	>0.1 NS
Eosinophiles# x10^6/l	0-0.7 0.16±0.14	0-0.7 0.15±0.12	>0.2 NS
Basophiles # x10^6/l	0-0.45 0.068±0.049	0-0.04 0.055±0.033	>0.1 NS

S: Significant p<0.05, NS: Non significant p>0.05.



	Neut #	Lymph #	Mono #	Eos #	Baso #
Exposed group	4.8	2.2	0.5	0.16	0.068
Non exposed	4.3	2	0.51	0.15	0.055

Fig. (1): The blood picture variables in studied groups.

Table (3): Blood picture variables in the studied groups as regard to age & sex.

	WBC x 10^9/L	RBC x 10^{12}/L	Hb g/dl	Ht %	Retic %	Differential count #: x10^9/L		
						Neut	Lymph	Mono
				Mean±SD				
Non exposed:								
I-M: <30Ys	6.2±2.1	5±1.2	14±1.7	44.3±4.4	1.2±0.2	3.5±1.3	2.1±0.1	0.5±0.2
II-F: <30Ys	6.9±3.4	4±0.7	11.2±0.8	34.2±3.1	1.0±0.7	3.7±2	1.7±0.3	0.49±0.1
III-M >30Ys	5.8±2.6	5.3±0.7	15±0.7	46.6±4.2	1.4±0.2	4.3±1.4	1.4±0.2	0.5±0.2
IV-F: >30Ys	6.4±2.7	4.3±0.8	12±1.2	38.3±2.6	0.7±0.3	4.4±2.4	1.8±0.6	0.4±0.1
Exposed:								
V-M: <30Ys	7.3±4.3	5.2±1.3	14±4 1.3	45.7±3.6	1.4±0.5	5.2±2.6	2.4±0.4	0.5±0.1
VI-F: <30Ys	8.4±3	3.8±1	11±0.7	35.7±3.5	1.5±0.4	7±2.8	2.2±0.4	0.5±0.2
VII-M >30Ys	7.4±1.8	5.3±0.9	15±1.2	48.6±4.8	1.4±0.3	6.4±1.8	2.5±0.5	0.4±0.2
VIII-F: >30Ys	7±2.3	4±0.8	12±1.7	34.4±3.6	1.5±0.4	6.4±3.5	2.4±0.3	0.5±-0.1
p-values:								
I versus V	NS	NS	NS	NS	NS	S*	NS	NS
II ver. VI	S*	NS	NS	NS	NS	HS*	NS	NS
III ver. VII	S*	NS	NS	NS	NS	NS	NS	NS
IV ver. VIII	NS	NS	NS	NS	S*	NS	NS	NS
V ver. VI	NS	NS	S*	S*	NS	NS	NS	NS
VII ver. VIII	NS	NS	S*	S*	NS	NS	NS	NS
V ver.VII	NS	NS	NS	NS	NS	NS	NS	NS
VI ver. VIII	NS	NS	NS	NS	NS	NS	NS	NS

NS: Non significant. S*: Significant. HS**: Highly significant. M: Male. F : Female.

Table (4): Blood platelets variables in the studied groups.

Platelet variables	Exposed group range Mean±SD	Non exposed group range Mean±SD	*p* value
Count x 10^9/L	177-447 305±72.4	212-397 276.03±48.4	<0.05 S*
MPV fl	7-15 9.5±1.8	7-12 8.36±1.02	>0.2 NS
Pct%	0.07-0.45 0.24±0.08	0.18-0.37 0.22±0.04	>0.3 NS
PDW (GSD)	14-23 16±1.5	14-19 16.4±1.11	>0.1 NS
Platelet activation by ADP/ collagen (closure time sec.)	124-135 132.3±2.6	143-148 145±4.5	<0.04 S*
Platelet activattion by EPI/collagen (closure time sec.)	145-152 146.4±3.3	148-159 154±3.7	<0.03 S*

*S: Significant. NS: Non significant.

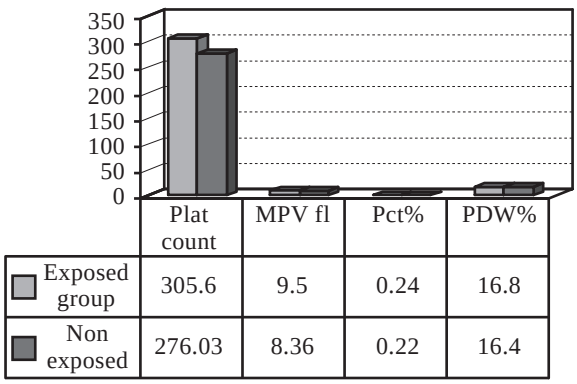


Fig. (2): Platelet variables in the studied groups.

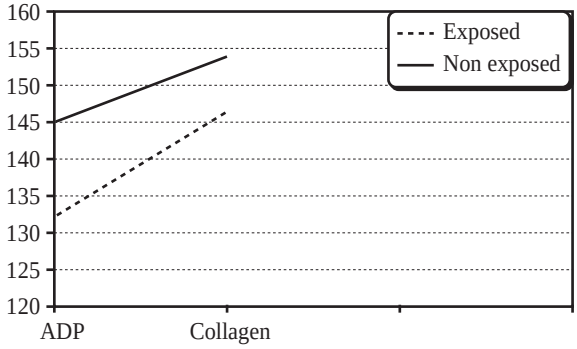


Fig. (3): Platelate activation in the studied groups.

Table (5): Platelet changes as regard to age & sex.

	Count x 10^9/L	MPV fl	PDW (GSD)	Plat aggr: ADP closure time (sec)	Plat aggr: collagen closure time (sec)
			Mean±SD		
Non exposed:					
I-M: <30Ys	260±54	8.2±1.1	16.6±2.3	145.4±4.6	152.2±4.2
II-F: <30Ys	340±66	10±1.6	17±1.5	145.3±5.2	148.3±5.7
III-M >30Ys	330±33	7.8±2.3	15±1.6	147.6±6.2	155.5±4.7
IV-F: >30Ys	350±65	9.9±3.2	16.4±2.4	144.3±4.3	157.5±3.6
Exposed:					
V-M: <30Ys	280±64	11.7±2.2	17.2±2.4	133.5±5.3	150.6±3.7
VI-F: <30Ys	390±43	13.4±1.1	18.6±2.1	126.2±2.5	146.2±5.2
VII-M >30Ys	340±36	10.2±2.5	16.7±1.7	134.4±3.7	151.4±6.2
VIII-F: >30Ys	378±65	12.3±1.9	17.2±2.8	129.2±2.5	148.3±6.3
p-values:					
I versus V	NS	S*	NS	S*	NS
II ver. VI	NS	S*	NS	S*	NS
III ver. VII	NS	NS	NS	S*	NS
IV ver. VIII	NS	NS	NS	S*	S*
V ver. VI	NS	NS	NS	S*	NS
VII ver. VIII	NS	NS	NS	NS	NS
V ver.VII	NS	NS	NS	NS	NS
VI ver. VIII	NS	NS	NS	NS	NS

NS: Non significant. S*: Significant. HS**: Highly significant. M: Male. F : Female.

Coagulation profile changes: The screening tests for haemostasis (PT, aPTT, TT) showed a significant decrease in clotting time of only a.P.T.T in exposed group versus non exposed control group (*p*-values <0.05). A significant increase was found in concentration of coagulation factors VIII and IX ($p<0.05$) and a highly significant increase in concentration of coagulation factors VII and vWF ($p<0.001$) when the exposed group was compared to non exposed group. FVIII/vWF ratio is significantly decreased in exposed group versus non exposed ($p<0.05$).

When the data were analysed as regard to age and sex, the results were: PT only showed a significant decrease in clotting times when the old exposed male group >30 ys was compared to non exposed old male group ($p<0.05$). a.P.T.T, T.T, FVIII represent a non significant variation ($p>0.05$). Coagulation factor VII represented a very characteristic finding that was significantly increased in concentration when both the exposed young male and female groups <30 ys old were compared to the non exposed same age and sex groups ($p<0.05$), also a significant increase was found when the exposed old male and female groups were compared to the same age and sex groups ($p<0.05$). Within the exposed subgroups a significant increase was found when the young male group was compared to young female group ($p<0.05$), also a significant increased was found when the old male group was compared to old female group ($p<0.05$). vWF was significantly increased when both the young exposed male and female groups were compared to the non exposed groups of the same age and sex, also when the old exposed male group was compared to non exposed old one ($p<0.05$).

A highly significant increase was found when the old exposed female group was compared to the old non exposed one ($p<0.001$). FIX was only significantly increased when the exposed young female group was compared to non exposed young one ($p<0.05$). Coagulation profiles are listed in Tables (6,7) & Figs. (4-8)

Inflammatory markers: Hyperfibrinogenaemia was observed in 8 cases (15.68%) of exposed group (fibrinogen level was >400 mg/L) and not observed in control group, there is a highly significant increased in fibrinogen level in exposed group versus control group (*p*-value <0.003). CRP level was increased in 21 cases (41.1%) of the exposed group, the level was more than 6 mg/L and in one case only (2.5%) of non exposed control group and showed a significant increase in exposed group versus non exposed control group (*p*-value <0.02). D-dimer level was more than 350 ug/dl in 16 cases of exposed group (31.3%), a highly significant increased in the level was observed in the exposed

group versus the control group (p-value <0.001), as regard to age and sex fibrinogen level was significantly increased when both exposed young male and female groups <30 ys old were compared to the non exposed groups of the same age and sex, also when the old exposed female group >30 ys old was compared to the non expose old one (p<0.05), a highly significant increase was found when the young exposed female group were compared to the young exposed males, also when the old exposed femalegroup was compared to the old exposed male group (p<0.005). CRP and D-dimer levels represent a highly significant increase when both exposed young male and female groups were compared to the same age and sex of non exposed groups, also when both the old exposed male and female groups were compared to the non exposed old groups of the same age and sex (p<0.005). A significant increase in the leveles of CRP and D-dimer was found when the young exposed female group was compared to the young exposed male group and when the young exposed female group was compared to the old exposed female group (p<0.05). No significant variation was found when the old exposed male and female groups were compared to each other and when the young and old male groups were compared to each other (p>0.05). Inflammatory markers were listed in Tables (8,9), Figs. (9-12).

Table (6): Coagulaton profile in the studied groups.

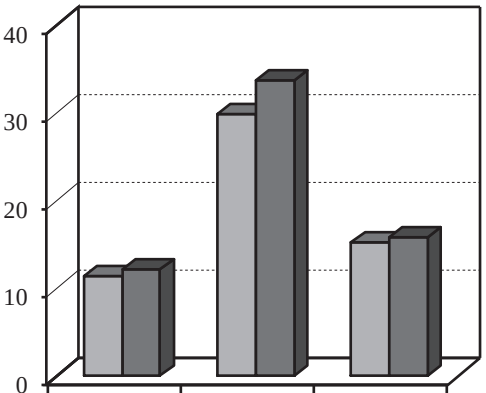
Varibles	Exposed group range Mean±SD	Non exposed group range Mean±SD	p values
PT sec.	9.9-13 11.3±0.72	11-13.9 12.17±0.8	>0.05 NS
aPTT sec.	22-37 29.8±4.3	23-42 33.7±3.7	<0.05 S*
TT sec	13.2-19 15.14±1.3	14-19 15.7±1.2	>0.05 NS
F VIII%	80-130 103±12.8	70-130 90.6±15.2	<0.05 S*
vWF%	110-156 133.2±10.3	70-120 89.05±16.98	<0.003 HS**
FVIII/vWF	0.72-0.83 0.77±0.08	1-1.08 1.01±0.89	<0.05 S*
F IX%	83-118 99.7±10.16	68-100 87.48±7.11	<0.05 S*
F VII%	120-210 144±11.9	65-100 82.87±8.96	<0.001 H*

NS : Non significant.
S* : Significant.
HS** : Highly significant.

Table (7): Coagulation profile as regard to age & sex.

	PT sec	aPTT sec	TT sec	F VII %	F VIII %	vWF %	FIX %
	Mean±SD						
Non exposed:							
I-M: <30Ys	11.8±0.5	31.2±3.2	14.4±1	85.6±8.7	80.5±10.6	75.5±7.5	105±10
II-F: <30Ys	12.2±1.3	34.4±2.7	16±1.3	70.4±.5.7	100±8.3	100.3±8.5	80.6±10.6
III-M >30Ys	12.4±0.4	30±4.2	15±0.9	95.6±7.4	120.4±11.6	90.5±11.3	85.7±12.6
IV-F: >30Ys	12.4±0.6	33.2±2.9	16.5±0.5	75.5±8.7	128.7±11.2	80.4±5.7	90±13.9
Exposed:							
V-M: <30Ys	10.4±0.8	28.5±3.2	14±1.3	200.5±11.8	95.6±10.2	120.4±10.6	110.6±8.6
VI-F: <30Ys	10.5±1.4	31.7±2.8	15±0.5	150.6±7.9	85.0±11.3	140.5±12.5	115.6±6.3
VII-M >30Ys	9.9±1.2	29.3±3.5	15±1.7	180.7±13.6	105.7±8.8	125.4±13.5	105.4±8.4
VIII-F: >30Ys	11±0.5	32.6±2.4	15±1.4	140.7±8.5	110.5±10.6	150.7±10.5	100.5±10.5
p-values:							
I versus V	NS	NS	NS	S*	NS	S*	NS
II ver. VI	NS	NS	NS	S*	NS	S*	S*
III ver. VII	S*	NS	NS	S*	NS	S*	NS
IV ver. VIII	NS	NS	NS	S*	NS	HS*	NS
V ver. VI	NS	NS	NS	S*	NS	NS	NS
VII ver. VIII	NS	NS	NS	S*	NS	NS	NS
V ver.VII	NS	NS	NS	NS	NS	NS	NS
VI ver. VIII	NS	NS	NS	NS	NS	NS	NS

NS: Non significant. S*: Significant. HS**: Highly significant. M: Male. F : Female.



	PT sec	aPTT sec	TT sec
Exposed group	11.3	29.8	15.14
Non exposed	12.17	33.7	15.7

Fig. (4): Coagulation profiles in the studied groups.

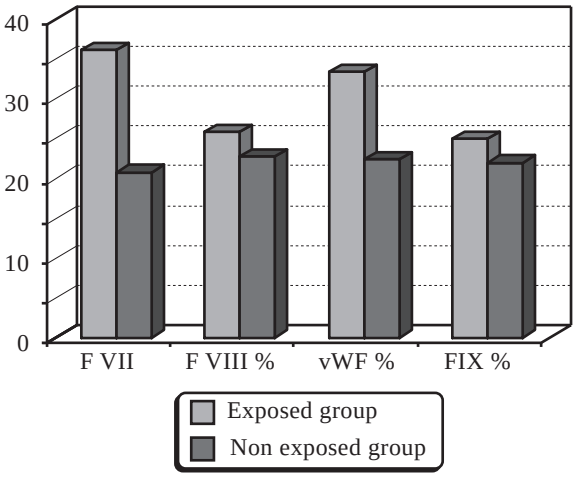
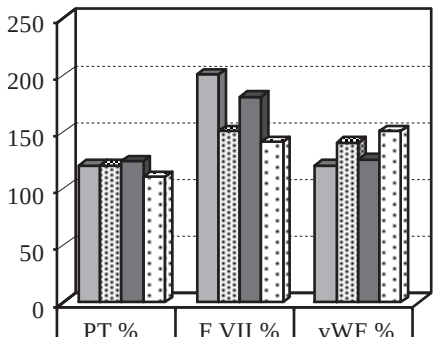


Fig. (5): Coagulation factors in the studied groups.



	PT %	F VII %	vWF %
Exp.M <30Ys	120	200.5	120.4
Exp.F <30Ys	120	150.6	140.5
Exp.M >30Ys	124	180.7	125.4
Exp.F >30Ys	110	140.7	150.7

Fig. (6): Coagulation variables in the exposed groups as regard to age & sex.

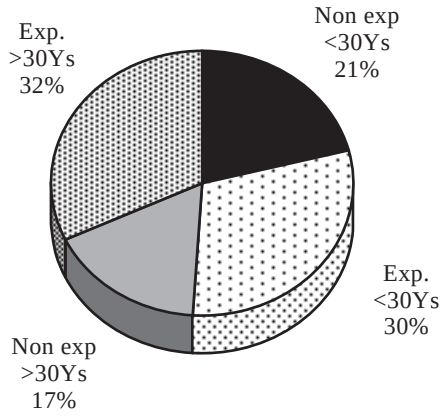


Fig. (7): vWF in the studied females groupes.

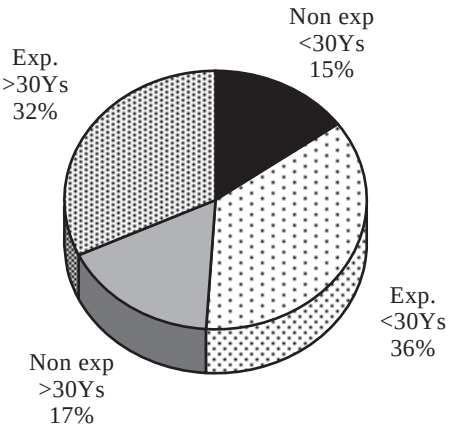


Fig. (8): Factor VII in the studied males groups.

Table (8): Inflammatory markers in the studied groups.

Varibles	Exposed group range Mean±SD	Non exposed group range Mean±SD	*p* values
Fibrinogrn mg/L	120-490 328.8±91	140-300 216.92±51.9	<0.003 HS**
CRP mg/L	0-36 12.11±6.52	0-12 3.7±2.21	<0.02 S*
D-dimer ug/dl	100-450 302.9±82.4	10-130 55.58±28.2	<0.001 HS**
vWF%	110-156 133.2±10.3	70-120 89.05±16.98	<0.003 HS*

S* : Significant.
HS** : Highly significant.

Table (9): Inflammatory markers as regard to age & sex.

	Fibrinogen mg/L	D-dimer ug/dl	CRP mg/L	vWF %
		Mean±SD		
Non exposed:	160.4±27.3	25.7±10.6	0	75.5±7.5
I-M: <30Ys	200.6±33.7	40.6±15.3	6.4±0.4	100.3±8.5
II-F: <30Ys	180.5±30.5	20±11.7	0	90.5±11.3
III-M >30Ys	260.4±35.4	65.7±14.8	0	80.4±5.7
IV-F: >30Ys		160±38.6		
Exposed:	195.5±34.5	210±37.8	4.2±2.6	110.6±8.6
V-M: <30Ys	320±40.4	180±28.9	16.3±4.4	115.6±6.3
VI-F: <30Ys	215±35.5	320±43.8	6.6±2.8	105.4±8.4
VII-M >30Ys	310±53.5		8.4±3.2	100.5±10.5
VIII-F: >30Ys		HS**		
p-values:	S*	HS**	HS*	S*
I versus V	S*	HS**	HS**	S*
II ver. VI	NS	HS**	HS**	S*
III ver. VII	S*	S*	HS**	HS*
IV ver. VIII	HS**	HS**	S*	NS
V ver. VI	HS**	NS	NS	NS
VII ver. VIII	NS	S*	NS	NS
V ver.VII	NS		S*	NS
VI ver. VIII				

NS: Non significant. S*: Significant. HS**: Highly significant.
M : Male. F : Female.

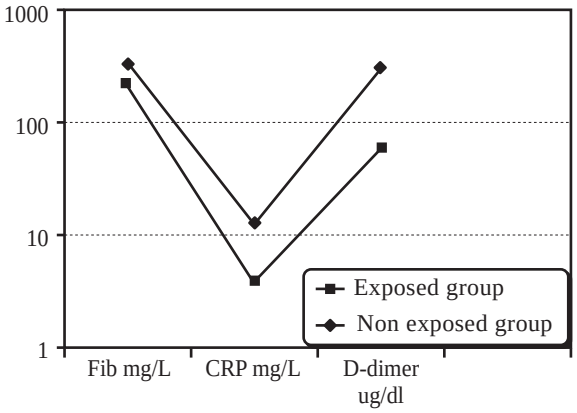


Fig. (9): Inflammatory markers in the studied groups.

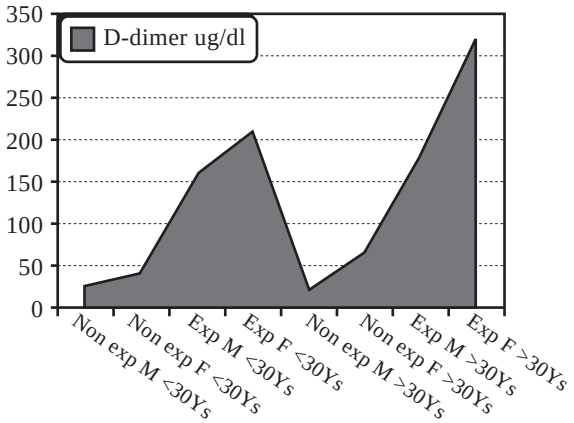
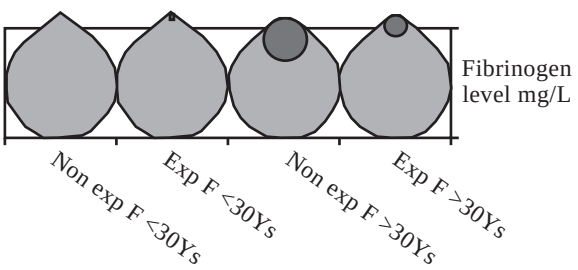


Fig. (10): D-dimer in the studied groups as regard to age & sex.



	Exp F <30Ys	Exp F >30Ys	Non exp F <30Ys	Non exp F >30Ys
Fibrinogen level mg/L	320	310	200.6	260.4

Fig. (11): Fibrinogen level in the studied females groups.

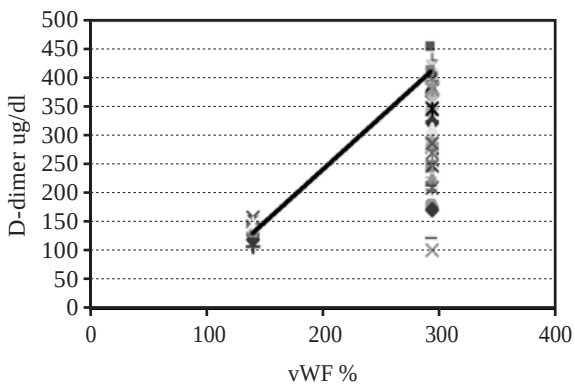


Fig. (12): Correlation between D-dimer & vWF in exposed group.

DISCUSSION

In the present study, exposure to P.M. of woodsmoke affects many of blood components that rinders most of them in the upper limit of normal or may exceed the upper normal range in certain condition, so these exposed groups are potentially at risk for thrombosis.

The total leukocytic count (WBCs) and specially, the absolute neutrophilic count is significantly increased, this is due to the release of inflammatory cytokines (IL-6) which affect the WBCs count and platelets count also was increased, this is in agreement with **[4]** who reported that bone marrow release of leukocytes and plateletes was an important component of the systemic inflammatory response.

MPV is significantly increased in the exposed groups, the newly released platelets are more large and giant than the old ones as reportedby.

The platelet activities are also significantly increased in the exposed group than the non exposed control group, this noticed by the decrease in the closure time of plateletes activation (by PF-100) by both ADP/collagen and EPI/ collagen, as the recently released platelets from the marrow megakaryocytes are more active as they contain most of its granule contents. Now there are two factors potentially increase the thrombotic tendency: the increase WBCs (neutrophiles) and increase both the platelet count and activities, these two components lead to the formation of platelet –leukocyte aggregates, which act as naidus for thrombus formation, this agrees with **[9]** who reported that a significant increase in leukocyte-platelet aggregates was found in women who used biomass as cooking fuel. In addition, they showed increased surface expression of CD11b/ CD18 in circulating neutrophiles and monocytes, also CD62P increased in expression.

The young exposed groups showed the more increase in WBCs and plateletes, as this young categories of people have more dueties and act more than the old one, so the exposure to pollutants of coocking fuel is more with more release of cytokines **[13]**.

Platelets activation by ADP/collagen in the PFA-100 showed more age and sex variation, than that by EPI/collagen, this is due to the fact that, ADP platelets aggregation occurs in two steps 1st receptors mediated process, 2nd the release of the endogenous ADP from the plateletes with subsequent aggregation, females below 30 years old whom are more exposed to pollutants and so the more cytokines released with more new platelets which contain all of its content granules so more activity, this is in accordance with **[14,15]**, who reported that amine polyester carbon particles affect platelets aggregation by ADP.

Coagulation profile response to P.M. of woodsmoke pollution affect aPTT that showed a significant shorting in clotting times this may be due to the increase concentration of the coagulation factors of the intrinsic pathway (F VIII, vWF, FIX) in the exposed groups, this not agree with who found that no association between pollution and a.P.T.T.

As regard to age and sex, P.T is significantly decreased in exposed males >30 years old group than the non exposed group, this may be due to the increase in concentration of factor VII **[16]**, who reported that factor VII was increased in response to P.M. and not agree with **[17]** who reported that no alteration in P.T and a.P.T.T with P.M. exposure.

vWF is highly significant increase in exposed females above 30 years old, as it is one of the acute phase reactant that is increases in response to the cytokines, this agree with **[5]**.

Inflammatory markers showed a highly significant incrase in plasma level of fibrinogen and D-dimer conentraion and significant increase in CRP level, these are acute phase reactant proteins that increase in inflammatory response as reported by **[12]**. Fibrinogen and D-dimer are rapid and early released inflammatory mediators than CRP (ultra sensitive CRP may be the more sensitive one as reported by **[18]**. Fibrinogen level is more affected by age and sex difference, it was increased more in exposed females groups below and above 30 years old than exposed males. This is similar to vWF response in female group, so the inflammatory response in females is exaggerated than male groups.

Conclusion:

* Exposure to P.M. of Woodsmoke is considered as a risk factor for thrombosis as it increase: WBCs (neutrophilic count), platelets

count and activities, coagulation factors (F VII, F VIII, vWF, F IX).

* Females below 30 years old are mor liable to thrombotic tendency as they are more exposed and have more activated platelets.

* Exposed males have the elevated level of factorVII (usually above 30 years old) are potentially at risk for thrombosis.

* D-dimer and CRP showed no sex variation, so can be used as as universal inflammatory marker with no sex affection D-dimer can be used as an indicator for thrombosis and inflammation irrespective to sex.

Recommendation:

Avoid the exposure to P.M of woodsmke as possible as we can by the use of protective mask and shorting the exposure times, increase the space between the exposure.

Alternate the traditional methods for cooking fuel to more automated machines.

Aknolowdgement:

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