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EVALUATION OF VON WILLEBRAND FACTOR AND D-DIMER CONCENTRATIONS AMONG SMOKERS IN PORT HARCOURT, NIGERIA

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ABSTRACT

This study evaluated Von Willebrand Factor(VWF) and D-dimer in Two hundred (200) male smokers within ages of 20-49 years and 138 age matched non- smoker controls in Port Harcourt, Rivers State. D-dimers and Von Willebrand Factor were determined using standard methods. The data were analyzed using Statistical Package for Social Sciences (SPSS) version 22. The result showed significant decrease ($P<0.05$) in VWF(ng/ml), concentration of smokers (1.31 ± 0.37) compared with non-smokers (4.68 ± 1.25) while there was significant increase ($P<0.05$) in D-dimer(pg/ml) concentrations of smokers (632.7 ± 105.5) compared with controls (126.4 ± 32.01). The study showed reduction in VWF with increase in D-dimer among smokers compared to their respective controls suggesting that smoking caused changes in Von Willebrand Factor(VWF) and D-dimer.

KEYWORDS: Von Willebrand Factor(VWF), D-dimer, smokers.

INTRODUCTION

Smoking, the act of inhaling and exhaling the fumes of burning plant material. A variety of plant materials are smoked, including marijuana and shisha, but the act is most commonly associated with tobacco as smoked in a cigarette, cigar, or pipe. Tobacco contains nicotine, an alkaloid that is addictive and can have both stimulating and tranquilizing psychoactive effects. The smoking of tobacco, long practiced by American Indians, was introduced to Europe by Christopher Columbus and other explorers. Smoking soon spread to other areas and today is widely practiced around the world despite medical, social, and religious arguments against it (Korenman, 2004). Smoking is one of the leading causes of death globally, accounting for over 6 million annually or at least 12 percent of deaths among people between age 30 and above (16 percent for men, 7 percent for women) (WHO, 2013). Smoking as a common lifestyle is seen as the single most preventable cause of cardiovascular diseases, which comprise a large number of conditions and are the number one cause of death globally, with an estimated 17.3–17.5 million deaths yearly (WHO, 2015). Smoking is also the leading cause of premature death from cardiovascular disease before age 70 years, estimated to be 5.9 million in 2013 (WHO, 2015). Such deaths deprive families of productive members and communities and economies of a productive workforce (Rigotti and Clair 2013).

Smoking induces a hypercoagulable state as a result of increased platelet aggregation, raised fibrinogen levels and polycythaemia (U.S. Department of Health and Human Services, 2010). D-dimer is a fibrin degradation product (FDP) a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. The antigen fibrin D-dimer is the primary enzymatic degradation product of cross linked fibrin by plasmin. Systemic values of D-dimer are an index of fibrin turnover in the circulation and a single measurement may be adequate to assess the fibrinolysis state (Raokin *et al*., 2014). Moderately elevated D-dimer reflects minor raising in blood coagulation, thrombin formation, and turnover of cross-linked intravascular fibrin and these increases may be relevant to coronary heart disease (Lowe & Rumley 1999). Increased levels of D-dimer indicate a global activation of hemostasis and fibrinolysis, and were associated with poor overall survival and increased mortality risk in cancer patients (Ay *et al*., 2012).

Damage to blood vessel walls exposes subendothelium proteins, most notably von Willebrand factor (vWF), present under the endothelium. vWF is a protein secreted by healthy endothelium, forming a layer between the endothelium and underlying basement membrane. When the endothelium is damaged, the normally-isolated, underlying vWF is exposed to blood and recruits Factor VIII, collagen, and other clotting factors. Circulating platelets bind to collagen with surface collagen- specific glycoproteinIa/IIa receptors. This adhesion is strengthened further by additional circulating proteins vWF, which forms additional links between the platelets glycoprotein Ib/IX/V and the collagen fibrils. These adhesions activate the platelets. Activated platelets

release the contents of stored granules into the blood plasma.

The aim of this study was to evaluate Von Willebrand Factor (VWF) and D-dimer of smokers in Port Harcourt, Rivers State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in Port Harcourt, capital of Rivers State, Nigeria. Port Harcourt is located at latitude $4{,}75^0$N and longitude 7.00^0E and lies along Bonny River in the Niger Delta. Population of the area is about 1,620,214 as at 2007 (Ibama and Wachukwu 2015). The Port Harcourt urban area is 2.7 million while greater Port Harcourt area is almost 2.7 million in population. Geography and infrastructure of Port Harcourt is highly congested.

Study Population

Based on convenient sampling method a total of Two hundred (200) adult male smokers aged between 20-49years old who smoked Cigarette, marijuana, Shisha or Cigar were recruited for this study and compared with age matched one hundred and thirty eight (138) non smokers to serve as control for this study. A structured questionnaire was administered to each participant to obtain their demographic information while Informed Consent was obtained from each participants. Inclusion Criteria include Males age range from 20 - 49 apparently healthy non smokers and Regular smokers of cigarette, shisha and marijuana and apparently healthy. Exclusion Criteria include Mixed water pipe and cigarette smokers, Age less than 18 or more than 50 years, Evidence or suspicion of cardiovascular diseases, hypertension, diabetes and recent surgery, Coagulation disorder (Hemophilia, protein C and S) and polythycaemia and Individuals who decline consents.

Sample Size

Sample Size of smokers in Rivers State was determined using the formular of Araoye (Araoye, 2004).

$$n = \frac{Z^2pq}{d^2}$$

Where,

n = sample size minimum

z = 95 % confidence interval

p = proportion of the largest population in Port Harcourt

d = with, degree of accuracy (95% interval) = 0.05%

∴ Using the formular the sample size is 185

Study Design

The study population consists of a total of Two hundred (200) adult male smokers aged between 20-49years old who smoked Cigarette, marijuana, Shisha or Cigar were recruited for this study and compared with one hundred and thirty eight (138) non smokers aged between 20-49years old. Both the smokers and non smokers were randomly sampled with their consent obtained and questionnaire administered to them in order to ascertain their demographic information, which were confidentially treated and maintained.

Sample Collection, Transportation, Processing and Preservation

Seven milliliter (7ml) of venous blood was collected from the antecubital fossa of each participants using a standard venipuncture technique. Four and half milliliter (4.5 ml) was dispensed into Sodium citrate anticoagulant bottle and properly mixed to ensure homogeneity and avoid clot formation while 2.5ml of blood was dispensed into labelled plain container for von Willebrand Factor analysis. The samples in the sodium citrate container were spun at 12,000rpm and plasma obtained, coagulation analyzer was used to measure the D-dimer concentration.

Determination of von Willebrand Factor was done based on Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human Von Willebrand Factor antibody. Von Willebrand Factor present in the sample is added to antibodies coated on the wells. And then biotinylated human Fibrinogen Antibody is added and binds to the Fibrinogen in the sample. Then Sreptavidin-HRP is added and binds to the Biotinylated Von Willebrand Factor antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and colour development in proportion to the amount of Von Willebrand Factor. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450nm.

Procedure: The number of strips to be used were determined and inserted into frames for use. Fifty microliter (50ul) of each standard was added to standard wells. Forty microliter (40ul) of sample and 10ul anti vWF were added to sample well. Fifty microliter (50ul) of streptavidin HRP was added to both standard and sample wells. The wells were mixed, cover and incubated at 37^OC for 60minutes. The plates were washed with wash buffer 5 times after incubation by soaking the wells with 0.35mls of wash buffer for 1minute for each wash. The plates were blotted onto paper towels. Fifty microliter (50ul)each of substrate solution A and substrate solution B was added to each well. The plates were covered and incubated for 10minutes at 37^OC in the dark. Fifty microliter (50ul) of stop solution was added to each well to change the colour from blue to yellow. The optical density of each well was read using a microplate reader at 450nm within 10minutes of adding stop solution and concentration of unknown determined from the plotted standard graph.

D-dimer Estimation is based on Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human D2D antibody. D2D present in the

sample is added to antibodies coated on the wells. And then biotinylated human D2D Antibody is added and binds to the D2D in the sample. Then Sreptavidin-HRP is added and binds to the Biotinylated D2D antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and colour development in proportion to the amount of D2D. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450nm.

Procedure: The number of strips to be used were determined and inserted into frames for use. Fifty microliter (50ul) of each standard was added to standard wells. Forty microliter (40ul) of sample and 10ul anti D2D were added to sample well. Fifty microliter (50ul) of streptavidin HRP was added to both standard and sample wells. The wells were mixed, cover and incubated at 37^{O}C for 60 minutes. The plates were washed with wash buffer 5 times after incubation by soaking the wells with 0.35mls of wash buffer for 1minute for each wash. The plates were blotted onto paper towels. Fifty microliter (50ul)each of substrate solution A and substrate solution B was added to each well. The plates were covered and incubated for 10minutes at 37^{O}C in the dark. Fifty microliter (50ul) of stop solution was added to each well to change the colour from blue to yellow. The optical density of each well was read using a microplate reader at 450nm within 10 minutes of adding stop solution and concentration of unknown determined from the plotted standard graph.

RESULTS

The D Dimer (pg/ml) of 632.7 ± 105.5 in smokers was significantly higher (P<0.05) than 126.4 ± 32.01 in control while the VWF (ng/ml) of 1.31 ± 0.37 in smokers was significantly lower(P<0.05) than 4.68 ± 1.25 as shown in table 1 below.

Table 1: D Dimers and VWF concentrations in smokers.

Parameters(Units)	Smokers	Control	p-value
D-dimer (pg/ml)	632.7 ± 105.5	126.4 ± 32.01	<0.0001 (S)
VWF (ng/ml)	1.31 ± 0.37	4.68 ± 1.25	<0.0001 (S)

There was significant difference (P<0.05) in VWF(ng/ml) of smokers of Cigar/Shisha, Cigar/Marijuana and Cigar (1.41 ± 0.33, 0.98 ± 0.32 and 1.31 ± 0.36) compared with the control(4.68 ± 1.25).Also there was significant difference(P<0.05) in D-dimer(pg/ml) of smokers of Cigar/Shisha, Cigar/Marijuana and Cigar(624.0 ± 134.4, 601.8±27.57 and 644.9±100.7) compared with the control (126.4 ±32.01) as shown in table 2.

Table 2: Combined Effects of Cigarette, Shisha and Marijuana on VWF and D-dimer in Smokers.

Parameters	Cigar/Shisha (A)	Cigar/Marijuana (B)	Cigar only (C)	Control (D)	F value	P value	TMC
VWF(ng/ml)	1.41 ± 0.33	0.98 ± 0.32	1.31 ± 0.36	4.68 ± 1.25	100.3	<0.0001	A – D = <0.001 B – D = <0.001 C – D = <0.001 Others = NS
D-dimer(pg/ml)	624.0 ± 134.4	601.8±27.57	644.9±100.7	126.4 ±32.01	304.3	<0.0001	A – D = <0.001 B – D = <0.001 C – D = <0.001 Others = NS

There were no significant difference (P<0.05) in the concentrations of D-dimer (pg/ml) and VWF (ng/ml) of different smokers in different age groups as shown in table 3.

Table 3: VWF and D-dimer concentrations based on Age of the Smokers.

Parameters	20-29 Years (A)	30-39 Years (B)	40-49 Years (C)	p-value	F-value	TMC Test
D-dimer (pg/ml)	639.4 ± 109.7	651.0 ± 115.5	606.8 ± 90.86	0.4772	0.751	All: NS
VWF (ng/ml)	1.45 ± 0.37	1.23 ± 0.33	1.23 ± 0.37	0.1233	2.189	All: NS

There was no significant difference in D-dimer (pg/ml) of 660.8 ± 136.5 in smokers on 1-10 Sticks per day and 615.0 ± 83.8 in smokers on greater than (>)10 Sticks per day as shown in table 4 below.

Table 4: VWF and D-dimer concentrations based on the Number of Cigarette Sticks Smoked Per Day.

Parameters/Units	1-10 Sticks	>10 Sticks	p-value
D-dimer (pg/ml)	660.8 ± 136.5	615.0 ± 83.8	0.1863 (NS)
VWF (ng/ml)	1.23 ± 0.33	1.32 ± 0.37	0.4056 (NS)

DISCUSSION

This present study investigated the changes or alterations in D Dimers and VWF concentrations of male smokers of cigarettes, shisha and marijuana or combined in Port Harcourt, Rivers State. Comparison of the values of these parameters were made with values obtained from subjects recruited as control who neither smokes, nor exposed to sources of carbon compounds or nicotine and/or components found in cigarettes.

The result of the study showed significant increase ($P<0.05$) in D-dimer with significant decrease ($P<0.05$) in von Willebrand factor concentrations among smokers compared to their respective non-smoker controls. Increased levels of D-dimer indicate a global activation of hemostasis and fibrinolysis, and were associated with poor overall survival and increased mortality risk in cancer patients (Ay *et al*., 2012). D-dimer is the end product of the breakdown of fibrin. It reveals the fibrinolytic activities in smokers.

Comparison was made in this study based on those who smoke shisha and marijuana, no comparison was made in respect to either of the former with cigarettes as all those smoking shisha and marijuana does smoke cigarettes, but some smokers neither smoke shisha nor marijuana. There was no statistical significance in all the studied parameters except von Willebrand factor based on those who smoke shisha when compared to those who smoke marijuana. In effect, it implies that irrespective of what is being smoked, there is no dominating effect of one over the other in terms raising the value of the parameters. Von Willebrand Factor was higher in those who smoke shisha than those who smoke marijuana, this may probably be as a result of the more cytotoxic effect of shisha over marijuana.

Analysis of the ages of the smokers recruited in the study was done and it was observed that the effect of smoking based on the age of those who smoke did not affect the outcome of the D Dimers and VWF as there were no statistical significant difference observed based on age classification. This is suggestive that age of the smokers does not have any effect on the D Dimers and VWF of male smokers studied.

The study did not report any difference in the D Dimers and VWF concentrations of subjects based on the number of cigarette sticks smoked per day. This is suggestive that number of cigarette sticks smoked per day neither increase nor decrease the D Dimers and VWF concentrations.

CONCLUSION

The study has shown changes in D Dimers and VWF of smokers.

REFERENCES

1. Araroye, M. O. (2004). Determination in Research Methodology with statistics for health and Social Sciences, *Scientific Research,* 115–121.
2. Ay, C., Dunkler, D., Pirker, R., Thaler, J., Quehenberger, P. & Wagner, O. (2012). High D-dimer levels are associated with poor prognosis in cancer patients. *Haematological,* 97(8): 1158-1164.
3. Ibama, B. & Wachukwu F. C. (2015). Settlement dynamics in the northern fringes of Port Harcourt Metropolis. *International Journal of Scientific and Technological Research*, 4(5): 34-43.
4. Korenman, S. G. (2004). Epidemiology of erectile dysfunction. *Endocrine*, 23: 87-93.
5. Lowe, G. D. & Rumley, A. (1999). Use of fibrinogen and fibrin D-dimer in prediction of arterial thrombotic events. *Circulation*, 82(2): 667-672.
6. Raokim, H., Prachee, M. & Sathe, D. (2014). Dinner in acute care. *International Journal of Critical Thesis and Injury Science*, 4(3): 229-235.
7. Rigotti, N. A. & Clair, C. (2013). Managing tobacco use: The neglected cardiovascular disease risk factor. *European Heart Journal*, 34(42): 3259–3267.
8. U.S. Department of Health and Human Service. (2010). The Health Consequences of Smoking: Cardiovascular Disease. A Report of the Surgeon General. Washington, DC: U.S. Department of Health and Human Services.
9. World Health Organisation (WHO) (2013). Report on the Global Tobacco Epidemic: Enforcing Bans on Advertising, Promotion AnsSponsorship. Geneva: WHO (2013).
10. World Health Organization (WHO) (2015). WHO Global Report: Mortality Attributable to Tobacco. 1365-2141 Geneva: WHO.