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Evaluation of Some Haemorheologic Parameters amongst Automotive Spray Painters in Port Harcourt, Nigeria

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Abstract

Background/Objectives: Automotive Spray-Painters are at a higher risk of exposure to hazardous chemicals such as polycyclic aromatic hydrocarbons – benzene inclusive, and heavy metals which may cause adverse health outcomes. The aim of the study was to evaluate effect of exposure to spray paints on some haemorheological parameters in individuals involved in automotive spray painting within Port Harcourt Metropolis. Method: This case control study recruited 52 participants: 30 automotive spray-painters and 22 controls. The study was carried out in Port Harcourt Metropolis, Nigeria. Venous blood samples were drawn from the participants (age 20 to 65 years) and examined for packed cell volume, erythrocyte sedimentation rate, fibrinogen and haemoglobin. Semi-structured questionnaire was used to obtain information from participants. Result: Participants from painting occupation had significantly higher ESR (p = 0.0450) as a result of increase in number of years in Automotive Spray-Painting. Increased ESR may be an indication of inflammation in the body. There was no statistical significance between Automotive Spray-Painters and control subjects when values of haemoglobin concentration, packed cell volume, fibrinogen concentration and erythrocyte concentration was compared in Automotive Spay-Painters, however, their values were increased than that control subjects at $p<0.05$. Conclusion: The study revealed that prolonged inhalation of benzene and other aromatic toxic compounds in paints caused slight increase in haemorheological parameters (PCV, HB, ESR and fibrinogen) of Automotive Spray-Painters due to exposure to paint fumes, a risk factor for inflammation of vital organs, venous thrombosis and thromboembolism; and predisposes Spray-Painters to many health disorders which can affect their lungs, heart, blood vessels, and ultimately the transport of oxygen to the brain.

Keywords: Haemorheology; Paints; Automotive Spray-Painters; Benzene Exposure.

1. Introduction

Occupational health is a neglected public health issue in developing countries of which Nigeria is one. This has exposed Spray-painters to various forms of hazards which have had negative consequences on their health due to the interactions of these substances with their body's physiological components, and ultimately their performance at work.

Occupational hazard is defined as the "potential risk to the health of a person emerging from an unhealthy environment" which is a significant public health issue. It can also be referred to as any activity, materials, processes or situation that is likely to cause an accident or disease at the work place. Although improvement in occupational health have been seen in many developed countries, however, the protection of workers from work-related disorders is not a priority in many developing countries, partly because several other health issues have competed with occupational health. This situation has existed for long owing to various socio-economic, cultural and political challenges which often make occupational health not prioritized [1].

Paints also known as surface coatings are multi-phase, complex and colloidal systems that are applied as continuous layer onto a surface [2]. Paint usually contains pigmented materials that enables it to be distinguished from clear films described as lacquers or varnishes, and they are used for surface coating and for aesthetics as well as surface protection. Automobile (Car) paint can enhance car appearance through the provision of colour and gloss and as well as protection of the car metal surfaces from corrosion. The pigmented particles have lead compounds in its composition, and organic solvents that includes both aliphatic and aromatic compounds, and also part of it are ethers, ketones, esters and ethers alcohol [3] In most workshops where spray-painting is carried out, the ambient environment is a reservoir of various contaminants that emerge from sources of chemicals being used while working resulting in direct exposure to these workers. Such exposures in chemical related occupations usually result from routine transportation, distribution, mixture of different paints, accidental spills, aerosol generation, leaching, and improper handling and use. Other way by which humans are exposed to these chemicals is by inhalation of toxic chemicals [4]. Many aromatic compounds that are used as solvents (ethyl benzene, xylene, toluene, etc., are components of petroleum products that has been implicated as carcinogens that can cause haematological malignancies [5], and benzene has been implicated as the main cause of health complications that could cause morbidity in automobile spray-painters [6].

In Port Harcourt, Nigeria where we have many artisans who are actively involved in spray-painting of automobiles, the use of protective gears like coverall, face shields and nose mask while working is not common, as most workers do not have these protective gears based on the fact that they are expensive. In their quest for maximum profit, they risk exposure to these chemical substances that can cause alterations in their haematological parameters. Most workers may spray up to three sport car per day, while the aerosols are generated during the spraying process, spray-painters without nose mask or face shield inhale the fumes, aerosols through the naso-oral route into their system where there is interaction with blood cells.

The major composition of the spray paints used by automobile spray painters in Port Harcourt are as follows: Acrylates (e.g. ethyl acrylate), Acrylic resins which serves as binders, Arsenic compounds (copper aceto-arsenate), which serves as antifouling agent, Gasoline which serves as the solvent, Aromatic hydrocarbons (benzene, toluene, xylene, trimethylbenzene) serving also as solvents, Lead compound (lead chromates, lead oxides, basic lead carbonates, and lead naphthenates) which serves as primers, pigments and also as driers.

Haemorheology has to do with blood flow, and this is very important for oxygen transport. Fibrinogen, erythrocyte sedimentation rate (ESR), packed cell volume (PCV) and haemoglobin concentration are important haemorhelogic parameters. Fibrinogen concentration, erythrocyte sedimentation rate, are important biomarkers used in assessing inflammation and blood viscosity in Automotive-Spray painters. The importance of haemoglobin estimation and determination of packed cell volume cannot be over emphasized in normal heamopoietic activities especially red blood cell production; as it will give the blood picture of the effect of the toxicity of benzene and other chemicals used in the manufacture of spray paints, which when inhaled and finally enters into the blood stream, causes destruction of blood cells, which will reflect in the values of these parameters. The aim of the study was to evaluate effect of exposure to spray paints on some haemorheological parameters in individuals involved in automotive spray painting within Port Harcourt Metropolis.

2. Material and Method

2.1. Study Design

This was a comparative case control study, carried out in Port Harcourt Metropolis, Port Harcourt City Local Government Area of Rivers State.

2.2. Study Area

The study was conducted in Port Harcourt Rivers State, Nigeria. Port Harcourt, the capital of Rivers State is located at latitude 4.78^{O} N and longitude 7.01^{O} E (4^{o} 47' 21" North, 6^{O} 59' 55" East) and lies along Bonny River in the Niger Delta. The analysis was carried out at Rivers State University, Haematology Laboratory, Department of Medical Laboratory Science, Port Harcourt. The study was caried out in June, 2019 to December, 2019.

2.3. Study Population

The research was carried out among automotive spray painters. Individuals not involved in spray painting, not exposed to sources of benzene, and were apparently healthy, were recruited as control subjects, with a total number of 52 male subjects, age ranging from 20-65 years.

2.4. Collection of Blood Samples and Storage

Venous blood (3 ml) was taken from a peripheral vein on the arm of each subject and immediately transferred into K_3-EDTA anticoagulant bottles at a concentration of 1.2mg/ml for haematological analysis and another 2 ml into a plain sterile bottle for the estimation of fibrinogen. The samples were left to stand at room temperature for

about 10 minutes and transferred to a cooler containing crushed ice for transportation to the laboratory, and later kept at room temperature for 30 minutes before analysis. Samples in the plain bottles were spun at 1000g for 15 minutes to obtain serum and was stored at -20^{0}C prior to being analyzed.

2.5. Determination of Haemoglobin Concentration

Method: Cyanmethaemoglobin Method. As described by Ochei and Kolhatkar [7].

Principle: It involves diluting blood sample with potassium cyanide, potassium ferricyanide and potassium dihydrogen phosphate. The ferricyanide forms methaemoglobin which is converted to cyanmethaemoglobin by the cyanide. The amount of cyanmethaemoglobin can be measured spectrophotometrically at a wavelength of 540nm with a spectrophotometer.

Procedure: Series of tubes was properly labelled for test sample, including a blank. 5ml of cyanmethaemoglobin reagent was pipette into each of the tubes, and 20ul of samples was added into the various test tubes labelled, excluding the blank tube. Tubes were allowed to stand for 10 minutes and observance of test was read using the spectrophotometer at 540nm wavelength with the blank solution.

2.6. Determination of Packed Cell Volume

Method: Microhaematocrit Method. As described by Cheesbrough [8].

Principle: Anticoagulated blood (EDTA) in a non-heparinized capillary tube is centrifuged using a microhaematocrit centrifuge at 12000rpm for 5 minutes to obtain constant packing of red cells. A small amount of plasma is trapped between the packed red cells with the use of a microhaematocrit reader, packed cell volume is read and results are expressed as L/L or percentage (%).

Procedure: A non-heparinized microhaematocrit tube was dipped into the sample container, the tube was filled to about two third (2/3). The microhaematocrit tube was sealed using a sealant and the tube was placed in the microhaematocrit centrifuge. It was spun at 1200rpm for 5 minutes, and after spinning results were read using the microhaematocrit reader with the base of the red cell on the '0' line and top of the plasma on the 100 line.

2.7. Determination of Erythrocyte Sedimentation Rate

Method: Westergren Method. As described by Cheesbrough [8].

Principle: Citrated blood is vertically positioned using Westergren pipette which is left undisturbed. Red cells aggregate and sediment through the plasma, the length of the column of clear plasma above the red cells and is measured in millimetre per hour (mm/hr).

Procedure: 0.4ml of trisodium citrate was pipetted into the Westergren bucket, together with 1.6ml of EDTA anticoagulated blood; proper mixing was done and the Westergren pipette was placed inside of the Westergren bucket, and was allowed to stand vertically undisturbed away from sunlight and vibration source. After one hour, results were read and expressed as mm/hour.

2.8. Determination of Fibrinogen

Method: Sandwich ELISA Method. Using Human Fibrinogen Elisa kit, Elabscience Biotech Co., Ltd, China; Catalog No: E-EL-H2193, Expiry Date: 2020-07-11

Principle: It makes use of the sandwich-enzyme linked principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human FG. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human FG and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human FG, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm ± 2 nm. The OD value is proportional to the concentration of Human FG. Calculation of the concentration of Human FG in the samples by comparing the OD of the samples to the standard curve.

Procedure: 100 µL of standards was added to each well labelled for standards and 100 µL of sample was also added to each well labelled for test. Both wells (samples and standards) was incubated for 90 minutes at 37°C. The liquid (samples and standards) was removed. 100 µL of biotinylated detection antibody was added and Incubated for 1 hour at 37°C. Each well was aspirated and washed for three times. 100 µL of horseradish peroxidase conjugate was added and Incubated for 30 minutes at 37°C. Aspiration was done on the various wells and washed for five times. 90 µL of substrate reagent was added and incubated for 15 minutes at 37°C. It was then followed by pipetting 50 µL of Stop Solution to each well. Absorbance of tests and standards was read at 450 nm immediately. Calculation of results was performed using BeerLambert's law.

$$\text{Concentration of Test} = \frac{\text{absorbance of test}}{\text{absorbance of standards}} \times \text{conc. of standards nearest to the Test}$$

3. Results

3.1. Demographic Details of Study Population

A total number of 52 subjects (males) were enrolled for this study. Of this number, 30 were test subject and the other 22 subjects served as control, within the age range of 20-65 years were recruited for the research in Port

Harcourt Metropolis, Rivers State, Nigeria within the period of July to December, 2019. The subjects were screened for Fibrinogen, Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV) and Haemoglobin (HB) Concentration were determined. Details are shown in Table I.

3.2. Comparison of Haemorheological Parameters in Study Population

In Spray Painters, the value of ESR indicated a statistically significant increase than the value obtained from control subjects at $p<0.05$. Other parameters showed no statistically significant differences. Details are shown in Table 2.

3.3. Comparison of Haemorheological Parameters in Spray Painters Based on Duration of Work

Among the cases however, only erythrocyte sedimentation rate (ESR) was statistically significant in sprayers of 0-9 years in the painting profession. Additionally, the other haematological parameters are not statistically significant. The PCV level in painters that have been exposed for over 10-19 years was slightly higher than other groups but not statistically significant. There was no significant difference in the values of HB concentration. Fibrinogen level was lowered in painters that have been exposed for 20-29 years. Details are shown in Table 3.

3.4. Comparison of Haemorheological Parameters in Spray Painters Based on Age Difference

ESR level is higher in individuals between the ages of 20-29 years and lowered in individuals between the age of 40-49 years. There was no significant change in all haemorheological parameters. Details are shown in Table 4.

Table-1. Demographic Details of Study Population

Subject	No. (% Distribution)
Tests	30 (57.69%)
Control	22 (42.31%)
Total	52 (100%)
Age Range	20-65 Years

Table-2. Comparison of Haemorheological Parameters in Study Population

Parameters	Control Mean ± SD	Test Mean ± SD	p-value	Inference
Haemoglobin (g/dl)	14.03 ± 1.324	14.11 ± 1.352	0.8269	NS
PCV (%)	42.45 ± 4.437	42.90 ± 3.517	0.6881	NS
ESR (mm/hr)	8.59 ± 5.59	20.03 ± 21.43	0.0183	S
Fibrinogen	592.5 ± 199.5	600.2 ± 107.5	0.8598	NS

Hb = haemoglobin; PCV = packed cell volume; ESR= erythrocyte sedimentation rate.

Table-3. Comparison of Haemorheological Parameters Using Analysis of Variance Based on Duration of Work

Parameters	0-9yrs (A) Mean ± SD	10-19yrs (B) Mean ± SD	20-29yrs (C) Mean ± SD	p-value	Fvalue	Remark	TMC-T
PCV (%)	43.44 ± 2.789	44.00 ± 1.789	43.58 ± 3.502	0.9371	0.065	NS	A vs B 0.9335 A vs C 0.9939 B vs C 0.9579
HB (g/dl)	14.58 ± 1.070	14.10 ± 1.106	14.24 ± 1.381	0.7303	0.319	NS	A vs B 0.7438 A vs C 0.8105 B vs C 0.9712
ESR	29.00 ± 26.50	10.33 ± 10.07	9.73 ± 2.028	0.0450	3.539	S	A vs B 0.0686 A vs C 0.0571 B vs C 0.0601
Fibrinogen	31.20 ± 6.323	31.28 ± 2.068	27.78 ± 5.724	0.4394	0.851	NS	A vs B 0.4264 A vs C 0.9399 B vs C 0.5593

Hb = haemoglobin; PCV = packed cell volume; ESR= erythrocyte sedimentation rate; TMCT = Tukey's multiple comparison test; NS = Non significance; S = Significance

Table-4. Comparison of Haemorheological Parameters Using Analysis of Variance Based on Age Difference

Parameters	20-29yrs (A) Mean ± SD	30-39yrs (B) Mean± SD	40-49yrs (C) Mean ± SD	p-value	Fvalue	Remark	TMCT
PCV (%)	42.00 ± 3.62	44.71 ± 3.49	42.50 ± 3.08	0.2498	1.467	NS	A vs B 0.2258 A vs C 0.9528 B vs C 0.4994
HB (g/dl)	13.94 ± 1.46	13.61 ± 1.21	14.88 ± 1.18	0.2324	1.548	NS	A vs B 0.8596

							A vs C 0.3351 B vs C 0.2306
ESR	27.33 ± 28.02	17.00 ± 7.28	10.33 ± 4.92	0.2369	1.526	NS	A vs B 0.5497 A vs C 0.2457 B vs C 0.8421
Fibrinogen	586.4 ± 92.32	602.2 ± 119.2	602.5 ± 148.9	0.9325	0.070	NS	A vs B 0.9492 A vs C 0.9529 B vs C >0.9999

Hb = haemoglobin; PCV = packed cell volume; ESR= erythrocyte sedimentation rate; TMCT = Tukey's multiple comparison test.

4. Discussion

The haemorheological parameters Heamoglobin (Hb), Packed Cell Volume (PCV), Fibrinogen (FIB) and Erythrocyte Sedimentation Rate (ESR) provide information on the general state of the blood flow, oxygen transport, as well as anaemia due to toxic chemicals of the subjects (auto spray-painters) used for this study. This study has demonstrated that exposure to spray paint fumes does not cause significant decrease in FIB values, Hb values and PCV of subjects exposed to spray paint fumes. The reason attributed to this when compared to control subjects may be as a result of the soot in the atmosphere in Port Harcourt that has been observed over a period of time starting from 2015 as reported by CNN correspondent [9]. Based on number of years (duration of exposure) there was significant decrease in the value of ESR as duration increases which is indicative of inflammation and a gradual adaptation to it as these individuals are exposed to paint fumes in addition to the soot, and the mechanism for this adaptation may be as a result of the body physiological processes to restore normal cellular activities through defensive adaptation that prevents these fumes and particles from getting into the lungs by mucus secretion [10].

No statistically significant difference was observed in PCV in this study, whereas Kamal and Malik [6] reported statistically significant increase in PCV values of painters compared to their control subjects. Their study and this study both showed that PCV was higher in Auto-Painters than in control, the reason for this may not be clear, however, their PCV levels are within normal ranges.

No statistically significant difference was observed in haemoglobin concentration as observed in this study, and this finding is antagonistic to that of Kamal and Malik [6] where they reported a decrease in haemoglobin concentration in spray painters compared to control subjects, and the reason for this disagreement is that the location of their study was not polluted with soot as it was the case in Port Harcourt.

Fibrinogen concentration in automobile spray-painters showed high concentration but without statistical significance. The measurement of fibrinogen concentration is critical because it is an acute phase reactant. The raised concentration of fibrinogen in these spray painters is actually indicative of the toxicity nature of the paints inhaled through the lungs and into the blood stream.

Duration of exposure and age of spray painters when HB, PCV and Fibrinogen were compared showed no statistically significant difference. However, ESR values in individuals who has more years of exposure shows high value which correlates to the degree of physiological inflammation.

The high level of HB, PCV, Fibrinogen and ESR in spray painters, though not significant compared to the control subjects whose values were lower, indicates that the viscosity of blood in spray-painters calls for concern and as such regular medical check-up is necessary to prevent health issues associated with increase in blood viscosity – which may predispose them to thrombotic events as increase in blood viscosity is a risk factor for thrombotic diseases. Soop, *et al.* [11], reported that blood viscosity is increased by elevated concentrations of acute phase reactants, thereby stimulating blood cells aggregation, increasing plasma viscosity also, which causes myocardial infarction, venous thrombosis and thromboembolism.

5. Conclusion

The slight increase in haemorheological parameters (PCV, HB, ESR and fibrinogen) of Automobile Spray-painters as a result of exposure to paint fumes is a risk factor for the inflammation of vital organs, venous thrombosis and thromboembolism, and as such a predisposition to many health disorders which can affect the lungs, heart, blood vessels, and ultimately the transport of oxygen to the brain. Therefore, good ventilation at work stations where spray-painting activities is taking place is important to ensure lower concentrations of benzene and other vapor-phase toxic chemicals are inhaled into the body. The use of nose mask and/or face shield is necessary to also reduce drastically the exposure to these toxic chemicals in paints.

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