Original Research Article

Assessment of some haematological, coagulation and immune parameters among male Oil Refinery workers in Port Harcourt, Nigeria.

ABSTRACT

Aim: The aim of this study was to assess of some hematological, coagulation and immune parameters among male oil refinery workers in Port Harcourt, Nigeria.
Study design: This study is a cross-sectional study.
Place and Duration of Study: Rivers State University Teaching Hospital (RSUTH), Port Harcourt, Shell Petroleum Development Company of Nigeria Limited and Modular Oil Refinery, Rivers State, between January 2021 and September 2021.
Methodology: A total of one hundred (100) subjects (50 oil and gas workers as test subjects and 50 non-oil and gas workers as control subjects), were enrolled in the study. The convenient sampling technique was employed in the study. Venous blood samples were collected from all subjects and tested for Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Fibrinogen concentration, Full Blood Count (FBC) (Haemoglobin (Hb), Packed Cell Volume (PCV), Total White Blood Cell (WBC) count, Lymphocyte, Neutrophils, monocytes and Platelet Count), CD4 cell count, CD3 cell count and CD8 cell count. Data generated were analyzed statistically using Graph-Pad Prism, Version 8.0.2.
Results: There was statistically significant decrease (*P<.001*) in Prothrombin time (PT) (11.55±0.73s), International Normalized Ratio (INR) (0.85±0.05), Fibrinogen (202.4±27.4mg/dl), Platelet count (185.6±37.1(10^3/µl)) and Neutrophils (46.6±6.4%) in oil refinery workers exposed to gas flare, while there was a statistical significant increase in APTT (31.8±4.15s), Hb (13.7±1.0g/dl), PCV (41.1±3.2%), Monocytes (8.4±3.0%) in subjects exposed to gas flare over control subjects with Prothrombin time(12.23±0.82s), INR(0.90±0.06), Fibrinogen(252.0±57.0mg/dl), platelet count(213.3±49.5(10^3/µl)) and Neutrophils(52.6±11.7%). Other parameters showed no statistical significant difference at P<0.05 in both Test and Control subjects. Comparison of the Mean ± Standard Deviation of the studied parameters in Test subjects based on Age using Analysis of Variance showed no statistically significant difference in all parameters at *P<.05*. Also, Comparison of the Mean ± Standard Deviation of the studied parameters in Test subjects based on Duration of Exposure using Analysis of Variance showed a significant decrease in CD8 cells as the years of exposure increase (2-5years exposure = 865±319, 6-10years exposure = 579±288, 11-20years exposure = 591± 286, F- Value = 3.869, P- Value = 0.0278).
Conclusion: In conclusion, based on the findings, duration of exposure can therefore be considered as a risk factor and age was considered not a risk factor as to cause any aberrations in the studied parameters.

Keywords: Haematological, coagulation, immune parameters, male Oil Refinery workers, Port Harcourt, Nigeria.

1. INTRODUCTION

Gas flaring is the process of burning-off associated gas from wells, hydrocarbon processing plants or refineries, either as a means of disposal or as a safety measure to relieve pressure [1]. It is now recognized as a major environmental problem, contributing an amount of about 150 billion m^3 of natural gas flared around the world, thus contaminating the environment with about 400mt Co_2 per year [2-3]. The world is currently facing global warming as one of its main issues. This problem can be caused by a rise in CO_2, CH_4 and other greenhouse gases (GHG) emissions in the atmosphere [2]

Environmental consequences associated with gas flaring have a considerable impact on local population, often resulting in severe health issues [4]. Pollutants of flare (such as benzene) have been reported to be poisonous, carcinogenic, causing blood abnormalities and immuno-toxic effects [4-5]. Owing to the several health challenges posed by individuals exposed to hydrocarbon gas flaring, it is therefore imperative to consider its effect on some clinical

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laboratory parameters such as immune parameters: CD4 cell count, CD3 cell count and CD8 cell count, Haematological parameters such as haemoglobin concentration, packed cell volume (PCV), total white blood cell count, differential lymphocyte count, monocytes, neutrophils and platelet count, Coagulation parameters such as prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen concentration. CD4+ T helper cells are white blood cells that are an essential part of the human immune system. They are often referred to as CD4 cells, T-helper cells or T4 cells. They are called helper cells because one of their main roles is to send signals to other types of immune cells, including CD8 killer cells, which then destroy the infectious particle. If CD4 cells become depleted, the body is left vulnerable to a wide range of infections that it would otherwise have been able to fight [6]. Cluster of differentiation 8 (CD8) is a trans-membrane glycoprotein that serves as a co-receptor for the T-cell receptor (TCR). Like the TCR, CD8 binds to a major histocompatibility complex (MHC) molecule, but is specific for the class 1 MHC protein [7]. There are two isoforms of the protein, alpha and beta, each encoded by a different gene. In humans, both genes are located on chromosome 2 in position 2P12. Cluster of differentiation 3 (CD3) is a protein complex and T cell receptor that is involved in activating both the cytotoxic T cells (CD8+ naïve T cell) and helper cells (CD4+ naïve T cell) [8].

Nigeria flares 17.2 billion m^3 of natural gas per year in conjunction with the exploitation of crude oil in Niger Delta [9]. These gases are mostly emitted in the Niger Delta area of Nigeria. Inhabitants of the region complain of health problems mainly respiratory tract diseases as well as damage to wild life vegetation [10]. Also, the knowledge that most human diseases and sufferings are sometimes related to the hazards of their work place meant that appropriate remedies to the situation would be possible only when these hazards are properly assessed, their very nature, extent and impacts firmly established [11]. This research is therefore necessitated in assessing the effect of gas flaring (if any) on some important haematological, immunological and coagulation laboratory parameters among oil and gas workers, and to ascertain the extent of deviation when compared to apparently healthy non-oil and gas workers.

2. MATERIALS AND METHODS

2.1 Study Design

This study is a cross sectional study carried out on oil and gas workers and non-oil and gas workers residing in Rivers State.

2.2 Study Area

This study was carried out in Port Harcourt, Rivers state, Nigeria. Rivers State is a state in the Niger Delta region of Nigeria. Its geographical coordinates lies along Latitude 4^0 44' 59 N and Longitude 6^0 49' 39 E. It was formed in the year 1967. The state capital, Port Harcourt, is a metropolis that is considered the commercial center of the Nigeria oil industry. Rivers State has a total area of 11,077Km^2 (4,277 sq. mi), making it the 26th largest state in Nigeria (Rivers State government website 2010). With a population of 5,198,716 as of the 2006 ensure, Rivers State is the 6th most populous state in the country. The state is particularly noted for its linguistic diversity, with 28 indigenous languages being said to be spoken in Rivers State (Rivers State government website 2010). The 26th largest state by area, Rivers State's geography is denominated by the numerous Rivers that flow through it, including the Bonny Rivers. The economy of Rivers State is dominated by the state's booming petroleum industry.

2.3 Study Population

A total of 100 male subjects were enrolled in this study. Subjects comprised of 50 oil and gas workers as test subjects and 50 non-oil and gas workers as control subjects. All the subjects enrolled in this study reside in Rivers state, and are Nigerians. A convenience sampling technique was adopted in this study. Questionnaire was administered to all subjects after obtaining their consent to participate in the study. The instrument for data collection was used to retrieve data such as Age, gender, duration of service (exposure), lifestyle, health issues, anticoagulant therapy medications and immune suppressive conditions.

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2.4 Selection Criteria

2.4.1 Inclusion Criteria

The following subjects were enrolled in the study: apparently healthy male individuals, workers in the oil and gas industry directly exposed to hydrocarbon gas flares, non-oil and gas workers not directly exposed to gas flares (those living in areas from gas flaring), Persons aged 26years and above, persons who had consented and answered the questionnaire tied to the study.

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2.4.2 Exclusion Criteria

The following subjects were excluded from the study: persons who did not give consent to nor answered the accompanied questionnaire for the study, persons who were younger than 26 years of age, persons on anti-coagulant therapy, and persons with immune suppressive conditions.

2.5 Samples Collection, Processing and Analysis

2.5.1 Sample Collection and Processing

6.5ml of venous blood was collected from each subject; 2.25ml of the blood sample was dispensed aseptically into 0.25ml of sodium citrate solution (109mmol/L) (making a 1:9 dilution), while the remaining 4.25ml of blood was dispensed into EDTA bottles. The samples collected into EDTA and sodium citrate bottles were carefully mixed using standardized mechanical mixer. The samples were transported immediately to the laboratory after collection in cooling box containing ice pack to maintain a room temperature condition. In the laboratory, blood samples in sodium citrate bottles were centrifuged at 2000g for 15minutes to obtain platelet poor plasma (PPP). Samples in EDTA bottles were analyzed immediately. Plasma samples obtained from sodium citrate bottles were analyzed immediately for prothrombin time and activated partial thromboplastin time. The remaining plasma sample was stored for fibrinogen analysis at a later date by freezing at -40^{o}C. The frozen samples were later thawed, mixed thoroughly but gently before analyzing.

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2.5.2 Sample Analysis

2.5.2.1 Estimation of Full Blood Count (FBC) Using Sysmex KX -21N Analyzer

Principle: Automated analyzer was used for assessment of full blood count using whole blood or pre dilute mode. 10µl of whole blood is aspirated through the sample probe into the sample rotor valve. 6µl of blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.994ml of diluents. At the same time 1.0ml WBC/ Haemoglobin (HGB) lyse is added to prepare 1:500 dilution sample. When the solution is made to react in this status for approximately 10 seconds, RBC is haemolysed and platelets shrink with WBC membrane held as they are. At same time, haemoglobin is converted into red coloured met-haemoglobin. Of the diluted/haemolysed sample in the WBC transducer chamber approximately 1.0ml is transferred to the HGB flow cell. 500µl of sample in WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC deflection method. In the HGB flow cell, 555nm wavelength beam irradiated form the light emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluents alone that was measured before addition of the sample, thereby calculating HGB (haemoglobin) value [12].

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Comment [H.O48]: replace 1.0ml by 1.0 ml

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2.5.2.2 Estimation of CD4/CD3 count using BD FACScount Automated CD4 Count

Principle: When whole blood is added to the reagents, fluorochrome-labeled antibodies. The reagents bind specifically to lymphocytes surface antigens, and a fluorescentnuclear dye binds to the nucleated blood cells. After a fixative solution is added to the reagent tubes, the sample is run on the instrument. During sample acquisition, the cells come in contact with the laser light, which cause the flourochrome-labeled cells and fluorescently dyed cells to fluoresce. The fluorescent light provides the information necessary for the instrument to identify and count the lymphocytes and CD4 lymphocytes. In addition, the reagent tubes also contain a known number of florescent reference beads. A precise volume of whole blood is stained directly is the reagent tube. The software automatically identifies lymphocyte

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population and calculates the CD4 count by comparing cellular events to bead events. The results are printed immediately after sample run (BD FACSCount users guide).

2.5.2.3 Determination of Prothrombin Time (PT) Using Helena C-1 Single-Channel Coagulometer

Principle: The one stage prothrombin time measures the clotting time of test plasma after the addition of thromboplastin reagent containing calcium chloride. The reagent supplies a source of tissue thromblastin, activating factor VII, and is therefore sensitive to all stage II and III factors. Deficiencies of stage I factors (VIII, IX, XI and XII) are not detected by the test [13].

2.5.2.4 Determination of the Activated Partial Thromboplastin Time (APPT) Using the Helena C-1 Single Channel Coagulometer.

Principle: The APTT test measures the clotting time of test plasma after the addition of APTT reagent, allowing an "activation time", followed by the addition of calcium chloride. Deficiencies of approximately 40% and lower of factors VIII, IX, XI and XII will result in a prolonged APTT. Heparin, in the presence of adequate amounts of AT-III will also result in a prolonged APTT [13]

2.5.2.5 Determination of Fibrinogen Concentration Using the Helena C-1 Coagulometer

The fibrinogent reagent utilizes the Clauss clotting method for the determination of plasma fibrinogen levels, where in excess Bovine Thrombin is used to clot diluted plasma. First, a standard curve is prepared using reference plasma of known fibrinogen content. When thrombin is added, the clotting time obtained is inversely proportional to the fibrinogen content. Next, patient plasma, at a dilution of 1:10, is clotted with thrombin and the resultant clotting time used to interpolate fibrinogen concentration from the standard curve [13].

3. RESULTS AND DISCUSSION

Flared gas is the most significant source of air emission from offshore oil and gas installations [14]. During most of these activities in the oil and gas industries, waste, either solid, liquid or gaseous forms are generated and discharged into the environment [15]. A cocktail of benzene and other toxic substances are emitted in these flares which have been considered harmful to humans, animals, plants and the entire physical environment [16].

Results from Table 2 revealed a statistical significant decrease in Prothrombin time, INR, Fibrinogen, platelets and neutrophils in subjects exposed to gas flares, while there was a statistical significant increase in APTT, haemoglobin, PCV, monocytes in subjects exposed to gas flares over control subjects. Other parameters (Total WBC, Lymphocytes, CD3 cell count, CD4 cell count and CD8 cell count) showed no statistically significant difference, all at *P<.05*.

In this study, the mean platelet count and neutrophils level of oil and gas workers (test subjects) showed a statistically significant decrease (*p=.002*) than that of the control subjects (Table 2). This result is in agreement with the result obtained by Egwurugwu et al. [17], who showed a statistically significant decrease in platelet count levels of subjects exposed to gas flare. This study also agrees with the findings from a similar study by Hanan et al. [18], who conducted a similar study on chronic benzene exposed workers, and revealed a significant decrease in platelet count levels of exposed workers when compared with controls. This study is however contrary to the findings of Ezejiofor, [19] and Christian et al. [20]. In a similar study of theirs, they observed no significant difference in the platelet count levels of exposed subjects to hydrocarbon products and the control counterparts. Also, the statistically significant decrease (*P=.002*) obtained in the levels of neutrophils of exposed subjects in this study (Table 2) is in agreement and support with a similar study conducted by Ezejiofor, [19], who reported a significant decrease in granulocyte levels of exposed subjects to petroleum products when compared to controls.

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Comment [H.O54]: replace *2.5.2.4 Determination of the Activated Partial Thromboplastin Time (APPT) Using the Helena C-1 Single Channel Coagulometer.* by 2.5.2.4 Determination of the Activated Partial Thromboplastin Time (APPT) Using the Helena C-1 Single Channel Coagulometer. (**Bold font**)

Comment [H.O55]: replace *2.5.2.5 Determination of Fibrinogen Concentration Using the Helena C-1 Coagulometer* by ***2.5.2.5 Determination of Fibrinogen Concentration Using the Helena C-1 Coagulometer*** (**Bold font**)

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Table 1 Demographic Details of Participants in the Study

Parameters Participant	Oil and gas workers Test Group (n=50)	Non-oil and gas workers Control Group (n=50)
Gender	Males	Males
Number of Subjects	50	50
Age Range (Years)	26-55	26-55
Residence	Rivers state	Rivers state
Nationality	Nigerian	Nigerian

The decrease seen in the levels of platelet count and neutrophils from this study indicates haematotoxicity of some blood indices to gas flare [4]. In addition, prolonged exposure to gas flares has been reported to cause marked decrease in some blood indices in humans [17].

In this study, a statistically significant decrease (*P<.001*) was seen in the prothrombin time (PT) and INR value of exposed subjects than their control counterparts (Table 2). The finding from this study is in agreement with the findings in a similar study conducted in Lombardia region in Italy by Baccarelli et al. [21], who reported a shorter prothrombin time and shorter INR values in individuals exposed to polluted air comprising of particulate matter, carbon monoxide, nitrogen oxide, sulphur dioxide and ozone (O_3) [21]. The decrease in the prothrombin time observed in this study (Table 2) is also in agreement with a similar study carried out by Bonzini et al. [22], on the effects of inhalable particulate matter on blood coagulation, who observed a shorter prothrombin time levels in exposed subjects.

This study also showed a statistically significant decrease (*P<.001*) in the fibrinogen level of subjects exposed to gas flare (oil and gas workers) than the controls (Table 2).

Table 2: Comparison of Mean ± Standard Deviation of the Studied Parameters in Test and Control Subjects.

Parameters	Control Mean ± SD	Test Mean ± SD	p-value	Remark
Prothrombin Time (s)	12.23 ± 0.82	11.55 ± 0.73	<0.0001	S
INR	0.90 ± 0.06	0.85 ± 0.05	<0.0001	S
APTT (s)	25.7 ± 5.56	31.8 ± 4.15	<0.0001	S
Fibrinogen (mg/dl)	252.0 ± 57.0	202.4 ± 27.4	<0.0001	S
WBC (10^3/µl)	5.2 ± 0.9	4.9 ± 1.0	0.1655	NS
Haemoglobin (g/dl)	11.7 ± 1.4	13.7 ± 1.0	<0.0001	S
PCV (%)	36.8 ± 3.6	41.1 ± 3.2	<0.0001	S
Platelets (10^3/µl)	213.3 ± 49.5	185.6 ± 37.1	0.0021	S
Lymphocytes (%)	42.8 ± 11.6	44.7 ± 6.0	0.3229	NS
Monocytes (%)	3.8 ± 1.9	8.4 ± 3.0	<0.0001	S
Neutrophils (%)	52.6 ± 11.7	46.6 ± 6.4	0.0022	S
CD3 count	1652 ± 410	1667 ± 743	0.8952	NS
CD4 count	959 ± 261	1026 ± 445	0.3660	NS

Table 2 Mean ± standard deviation of comparison the studied parameters in test and control subjects.

Parameters	Control	Test	p-value	Significant
Prothrombin Time (s)	**12.23^A ± 0.82**	**11.55^B ± 0.73**	**<0.0001**	**
INR	**0.90^A ± 0.06**	**0.85^B ± 0.05**	**<0.0001**	**
APTT (s)	**25.7^B ± 5.56**	**31.8^A ± 4.15**	**<0.0001**	**
Fibrinogen (mg/dl)	**252.0^A ± 57.0**	**202.4^B ± 27.4**	**<0.0001**	**
WBC (10^3/µl)	**5.2 ± 0.9**	**4.9 ± 1.0**	**0.1655**	**NS**
Haemoglobin (g/dl)	**11.7^B ± 1.4**	**13.7^A ± 1.0**	**<0.0001**	**
PCV (%)	**36.8^B ± 3.6**	**41.1^A ± 3.2**	**<0.0001**	**
Platelets (10^3/µl)	**213.3^A ± 49.5**	**185.6^B ± 37.1**	**0.0021**	**
Lymphocytes (%)	**42.8 ± 11.6**	**44.7 ± 6.0**	**0.3229**	**NS**
Monocytes (%)	**3.8^B ± 1.9**	**8.4^A ± 3.0**	**<0.0001**	**S**
Neutrophils (%)	**52.6^A ± 11.7**	**46.6^B ± 6.4**	**0.0022**	**S**
CD3 count	**1652 ± 410**	**1667 ± 743**	**0.8952**	**NS**
CD4 count	**959 ± 261**	**1026 ± 445**	**0.3660**	**NS**

A and B: Means in the same raw having different superscripts differ significantly at (P<0.01)
NS Not significant at (P<0.05).

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This finding is contrary to the findings Baccarelli et al. [21], who found no consistent relations between plasma fibrinogen level and exposure to polluted air comprising of CO, nitrogen oxides, SO_2 and ozone (O_3): these being constituents of gas flares also. The decrease in the prothrombin time, INR (International Normalized ratio) and fibrinogen levels obtained in this study may reflect gas flare exposure-related changes in human blood coagulation (Hypercoagulability tendencies). Prothrombin time (PT) measures the formation of the fibrin clot through the activity of the extrinsic and common coagulation pathways, which involves the interaction of tissue factor and activated Factor VII in addition to FX and FV, prothrombin and fibrinogen. Prothrombin Time is shortened in inflammatory condition or arterial thrombotic tendency [23]. Also, air contamination provokes oxidative stress, systemic inflammation and autonomic nervous system in balance that subsequently induce endothelial dysfunction and vasoconstriction leading to increased blood pressure [24].

Table 3: Comparison of Mean ± Standard Deviation of the Studied Parameters in Test Subjects Based on Age Using Analysis of Variance

Parameters	26-35 Years Mean ± SD	36-45 Years Mean ± SD	46-55 Years Mean ± SD	F-value	p-value
PT (s)	11.2 ± 0.8	11.5 ± 0.7	11.6 ± 0.7	0.8927	0.4164
INR	0.83 ± 0.06	0.86 ± 0.05	0.86 ± 0.05	0.9191	0.4059
APTT (s)	33.0 ± 5.3	32.3 ± 3.8	30.7 ± 3.9	1.192	0.3128
Fibrinogen (mg/dl)	189.4 ± 30.4	202.0 ± 25.9	208.8 ± 27.5	1.419	0.2520
WBC (103/µl)	5.2 ± 1.4	4.8 ± 0.9	5.0 ± 0.9	0.3689	0.6935
Hb (g/dl)	13.8 ± 1.3	13.7 ± 1.1	13.6 ± 0.8	0.1504	0.8607
PCV (%)	41.7 ± 4.2	41.0 ± 3.5	41.0 ± 2.5	0.1521	0.8593
Platelets (103/µl)	174 ± 44	190 ± 34	184 ± 38	0.5859	0.5606
Lymphocytes (%)	43.2 ± 6.8	44.7 ± 5.3	45.3 ± 6.6	0.3393	0.7140
Monocytes (%)	7.7 ± 4.0	8.3 ± 2.8	9.0 ± 2.7	0.5386	0.5871
Neutrophils (%)	49.0 ± 8.2	46.9 ± 5.7	45.2 ± 6.5	1.004	0.3741
CD3 count	2120 ± 920	1645 ± 683	1496 ± 694	2.058	0.1391
CD4 count	1248 ± 565	1035 ± 414	915 ± 412	1.598	0.2131
CD8 count	871 ± 364	621 ± 281	580 ± 297	2.745	0.0746

Table 3 Mean ± standard deviation of comparison of the studied parameters in test subjects based on age using analysis of variance

Parameters	26-35 Years	36-45 Years	46-55 Years	F-value	P-value	Signif
PT (s)	11.2 ± 0.8	11.5 ± 0.7	11.6 ± 0.7	0.8927	0.4164	NS
INR	0.83 ± 0.06	0.86 ± 0.05	0.86 ± 0.05	0.9191	0.4059	NS
APTT (s)	33.0 ± 5.3	32.3 ± 3.8	30.7 ± 3.9	1.192	0.3128	NS
Fibrinogen (mg/dl)	189.4 ± 30.4	202.0 ± 25.9	208.8 ± 27.5	1.419	0.2520	NS
WBC (103/µl)	5.2 ± 1.4	4.8 ± 0.9	5.0 ± 0.9	0.3689	0.6935	NS
Hb (g/dl)	13.8 ± 1.3	13.7 ± 1.1	13.6 ± 0.8	0.1504	0.8607	NS
PCV (%)	41.7 ± 4.2	41.0 ± 3.5	41.0 ± 2.5	0.1521	0.8593	NS
Platelets (103/µl)	174 ± 44	190 ± 34	184 ± 38	0.5859	0.5606	NS
Lymphocytes (%)	43.2 ± 6.8	44.7 ± 5.3	45.3 ± 6.6	0.3393	0.7140	NS
Monocytes (%)	7.7 ± 4.0	8.3 ± 2.8	9.0 ± 2.7	0.5386	0.5871	NS
Neutrophils (%)	49.0 ± 8.2	46.9 ± 5.7	45.2 ± 6.5	1.004	0.3741	NS
CD3 count	2120 ± 920	1645 ± 683	1496 ± 694	2.058	0.1391	NS
CD4 count	1248 ± 565	1035 ± 414	915 ± 412	1.598	0.2131	NS
CD8 count	871 ± 364	621 ± 281	580 ± 297	2.745	0.0746	NS

NS Not significant at (P<0.05).

In this study, a statistically significant increase (*p<.001*) was seen in the APTT levels of exposed subjects to gas flares than that of their control counterparts (Table 2). This finding is however contrary to the findings of Baccarelli et al. [21] who in a similar study found no association (no statistically significant difference) in the APTT level of subjects exposed to polluted air of particulate matter (PM), CO, SO_2 and ozone (O_3) and NO_2. This is also contrary to the results of Bonzini et al. [22], who in a similar study found no significant change

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Comment [H.O80]: replace *(p<.001)* by *(P<0.001)*

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in the APTT levels of steel plant production workers with well characterized exposure to particulate matter (flare pollutants).

In this study, a significant increase (*P<.001*) was observed in the Haemoglobin (HB) and Packed cell volume (PCV) levels of the test subjects than that of the controls (Table 2). The result from this study is contrary to the findings of Adienbo and Nwafor, [25], who reported a decrease in the Haemoglobin and PCV levels of subjects with prolonged exposure to gas flare in Niger Delta Region of Nigeria. Similarly the result from this study is also contrary to the findings of Ezejiofor, [19] and Christian et al. [20], who from a similar study on petroleum oil workers reported no statistically significant difference in the Haemoblobin and Packed cell volume levels of exposed workers and their control counterparts. The finding from this study is also contrary to the result obtained by Hanan et al. [18], who reported in a similar study, significant decrease in the Haemoblobin level of benzene (Hydrocarbon product) exposed workers when compared with controls. The increase in the Haemoglobin and Packed cell volume levels of the oil and gas workers in relation to their control counterparts may be due to the robust welfare of workers particularly in the oil and gas sector compared to other sectors of the Nigerian economy. Similar to Haemoglobin, the result in this study also showed a statistically significant increase (*P<.001*) in the mean monocyte level of the test subjects than that of the controls (Table 2). This is contrary to the result obtained by Hanan et al. [18], who reported a significant decrease in the monocyte level of exposed workers than that of the controls. The increase in the level of monocyte seen in test subject may be pointing as a marker towards an ongoing and unidentified inflammatory process resulting from exposure to hydrocarbon gas flare [26].

The result from Table 2 showed that there was no statistically significant difference (*P<.05*) in the total WBC count of the test subjects and control subjects. The result from this study is in agreement with that of Ezejiofor, [19], who in his study reported that total WBC count showed no appreciable difference in oil workers compared with those of the non-oil workers. This however is contrary to the finding of Adienbo and Nwafor, [25] and Egwurugwu et al. [17], who both reported an increase in the total WBC count of oil and gas workers than their control counterparts. The result from this also disagrees with that of Hanan et al. [18], who reported a decrease in the WBC count of exposed workers in relation to their control counterparts.

Comment [H.O82]: replace *(P<.001)* by *(P<0.001)*

Comment [H.O83]: delete ,

Comment [H.O84]: delete ,

Comment [H.O85]: delete ,

Comment [H.O86]: delete ,

Comment [H.O87]: delete ,

Comment [H.O88]: replace *(P<.001)* by *(P<0.001)*

Comment [H.O89]: delete ,

Comment [H.O90]: adding (2)

Comment [H.O91]: replace (*P<.05*) by *(P<0.05)*

Comment [H.O92]: delete ,

Comment [H.O93]: delete ,

Comment [H.O94]: delete ,

Comment [H.O95]: delete ,

Table 4: Comparison of Mean ± Standard Deviation of the Studied Parameters in Test Subjects Based on Duration of Exposure Using Analysis of Variance

Comment [H.O96]: I think that rewriting this Table (4) as the following

Parameters	2-5yrs (x) Mean ±SD	6-10yrs (y) Mean ±SD	11-20yrs (z) Mean ±SD	F-value	p-value	Remark
PT (s)	11.3 ± 0.7	11.5 ± 0.7	11.6 ± 0.7	0.5030	0.6080	ALL $^{(NS)}$
INR	0.84 ± 0.05	0.86 ± 0.05	0.86 ± 0.05	0.4794	0.6221	ALL $^{(NS)}$
APTT (s)	32.7 ± 4.7	33.0 ± 3.3	30.1 ± 4.2	2.852	0.0678	ALL $^{(NS)}$
Fibrinogen (mg/dl)	198 ± 32	202 ± 25	204 ± 27	0.1420	0.8680	ALL $^{(NS)}$
WBC (10^3/µl)	5.1 ± 1.3	4.8 ± 0.9	4.9 ± 0.9	0.1596	0.8529	ALL $^{(NS)}$
Hb (g/dl)	13.8 ± 1.4	13.7 ± 0.8	13.6 ± 1.1	0.1578	0.8545	ALL $^{(NS)}$
PCV (%)	41.5 ± 4.4	41.1 ± 2.6	40.9 ± 3.2	0.1119	0.8944	ALL $^{(NS)}$
Platelets (10^3/µl)	170 ± 37	191 ± 35	188 ± 38	1.127	0.3327	ALL $^{(NS)}$
Lymphocytes (%)	43.9 ± 5.9	43.7 ± 5.1	46.1 ± 6.8	0.9376	0.3988	ALL $^{(NS)}$
Monocytes (%)	8.5 ± 3.6	8.4 ± 2.8	8.4 ± 2.9	0.0051	0.9950	ALL $^{(NS)}$
Neutrophils (%)	47.4 ± 7.4	47.8 ± 5.8	44.9 ± 6.4	1.119	0.3352	ALL $^{(NS)}$
CD3 count	2128 ± 840	1521 ± 632	1554 ± 721	2.940	0.0627	ALL $^{(NS)}$
CD4 count	1263 ± 531	955 ± 360	963 ± 447	2.098	0.1341	ALL $^{(NS)}$
CD8 count	865 ± 319	579 ± 288	591 ± 286	3.869	0.0278	XvsY $^{0.0344}$ XvsZ $^{0.0466}$ YvsZ $^{0.9945}$

Table 4 Mean ± comparison of standard deviation of the studied parameters in test subjects based on duration of exposure using analysis of variance

Parameters	2-5yrs (x)	6-10yrs (y)	11-20yrs (z)	F-value	p-value	Significant
PT (s)	11.3 ± 0.7	11.5 ± 0.7	11.6 ± 0.7	0.5030	0.6080	NS
INR	0.84 ± 0.05	0.86 ± 0.05	0.86 ± 0.05	0.4794	0.6221	NS
APTT (s)	32.7 ± 4.7	33.0 ± 3.3	30.1 ± 4.2	2.852	0.0678	NS
Fibrinogen (mg/dl)	198 ± 32	202 ± 25	204 ± 27	0.1420	0.8680	NS
WBC (10^3/µl)	5.1 ± 1.3	4.8 ± 0.9	4.9 ± 0.9	0.1596	0.8529	NS
Hb (g/dl)	13.8 ± 1.4	13.7 ± 0.8	13.6 ± 1.1	0.1578	0.8545	NS
PCV (%)	41.5 ± 4.4	41.1 ± 2.6	40.9 ± 3.2	0.1119	0.8944	NS
Platelets (10^3/µl)	170 ± 37	191 ± 35	188 ± 38	1.127	0.3327	NS
Lymphocytes (%)	43.9 ± 5.9	43.7 ± 5.1	46.1 ± 6.8	0.9376	0.3988	NS
Monocytes (%)	8.5 ± 3.6	8.4 ± 2.8	8.4 ± 2.9	0.0051	0.9950	NS
Neutrophils (%)	47.4 ± 7.4	47.8 ± 5.8	44.9 ± 6.4	1.119	0.3352	NS
CD3 count	2128 ± 840	1521 ± 632	1554 ± 721	2.940	0.0627	NS
CD4 count	1263 ± 531	955 ± 360	963 ± 447	2.098	0.1341	NS
CD8 count	865 ± 319	579 ± 288	591 ± 286	3.869	0.0278	XvsY$^{0.0344}$ XvsZ$^{0.0466}$ YvsZ$^{0.9945}$

NS Not significant at (P<0.05).

Table 2 Also showed that there is no statistically significant difference (P<.05) in the lymphocyte value of subjects exposed to gas flare over control subjects. This result is contrary to that of Ezejiofor, [19], who reported in a similar study, a significant increase in the lymphocytes value of exposed subjects over their control. This study is also contrary to that of Hanan et al. [18], who reported a significant decrease in the lymphocytes of subjects exposed to hydrocarbon products over their controls.

Results from Table 2 showed no statistically significant difference (*P<.05*) in the mean immune parameters: CD3 count, CD4 count, CD8 count of subjects exposed to gas flares over control subjects. The result obtained in this study is contrary to the findings of Christopher and AsuQuo, [27], who in a similar study on gasoline station workers exposed to benzene, reported a significant decrease in the CD4, and CD4/CD8 ratio of exposed workers over their controls. Similarly, this is contrary to the findings of Hanan et al. [18], who in a similar study reported a decrease in CD3 cells, CD4 cells, CD4:CD8 ratio, and a significant increase in CD8 cells of workers exposed to hydrocarbon product (such as benzene) over their control subjects. The non-significant difference in the mean CD4, CD3 and CD8 count in the test and control subjects explains the healthy state of the immune system of the studied group as at the time of this research.

Table 3 showed comparison of Mean ± Standard Deviation of the studied parameters in test subjects based on Age using Analysis of Variance. Based on the ages of the subjects exposed to gas flares, there was no statistically significant difference in all parameters at *P<.05*. This implies that age is not a risk factor in assessing the effect(s) of hydrocarbon gas flaring on exposed subjects. This is contrary to the views of Ezejiofor, [19], who suggested that Age have a strong impact in defining the pattern of variations observed in the haematological indices among oil workers exposed to petroleum products in a petroleum refining and distribution industry.

Table 4 showed comparison of Mean ± Standard Deviation of the studied parameters in Test subjects Based on Duration of Exposure using Analysis of Variance. Based on duration of exposure to hydrocarbon gas flare, the Results of Table 4 revealed only a statistically significant decrease in CD8+ cells as the number of years of exposure increases. Other parameters showed no statistically significant difference at *P<.05*. This implies that the immunotoxicity may be a risk factor over long term exposure to hydrocarbon gas flares [27]. The decline in the number of the cytotoxic Killer T-cells (CD8+ cells) based on duration of exposure to gas flare as observed in this study reveals that the cytotoxic cells population of the T- lymphocytes may have been engaged in combat conditions over time, eliminating toxic substances which may have gained entrance into the body over time by reason of exposure.

Comment [H.O97]: Correcting Table 4 …. This forma is suitable (It Made by the reviewer)

Comment [H.O98]: replace (P<.05) by (P<0.05)

Comment [H.O99]: delete ,

Comment [H.O100]: delete ,

Comment [H.O101]: replace (P<.05) by (P<0.05)

Comment [H.O102]: replace , by and

Comment [H.O103]: delete ,

Comment [H.O104]: delete ,

Comment [H.O105]: delete ,

Comment [H.O106]: delete ,

Comment [H.O107]: delete ,

Comment [H.O108]: replace Standard Deviation by standard deviation

Comment [H.O109]: replace Age using Analysis of Variance by age using analysis of variance

Comment [H.O110]: replace *P<.05* by *P<0.05*

Comment [H.O111]: delete ,

Comment [H.O112]: delete ,

Comment [H.O113]: replace Standard Deviation by standard deviation

Comment [H.O114]: replace Test by test

Comment [H.O115]: replace Based on Duration of Exposure using Analysis of Variance by based on duration of exposure using analysis of variance

Comment [H.O116]: replace Results by results

Comment [H.O117]: Please 2 back space before revealed

Comment [H.O118]: Replace *P<.05* by *P<0.05*

4. CONCLUSION

In conclusion, based on the findings, duration of exposure can therefore be considered as a risk factor and age was considered not a risk factor as to cause any aberrations in the studied parameters.

ACKNOWLEDGEMENTS

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Samples for this study were obtained based on informed consent of the participants enrolled in the study. Ethical approval was obtained from the Rivers state Health Management Board, Port Harcourt, Nigeria

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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