Assessment of Interleukin 6 and C-reactive Protein as Inflammatory Markers among Patients with Substance Use Disorder in Federal Neuropsychiatric Hospital, Kaduna

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Authors' contributions

This work was carried out in collaboration among all authors. Author SYO designed the study, managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Authors BOPM and AJY managed the analyses of the study. Authors ORO and AAE performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This research aimed to determine the relationship between substance use disorder (SUD) and the biomarkers of inflammation: (C-reactive protein: CRP and Interleukin-6: IL-6) to investigate inflammatory reaction among SUD patients.
Study Design: This is a cross sectional comparative study.
Place and Duration of Study: This study was conducted at Federal Neuro-Psychiatric Hospital (FNPH) Kaduna, North-western Nigeria which serve as a referral center for patients with psychiatric disorders from all the North Western states and the Federal Capital Territory, Abuja between August 2018 and August 2019.

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Methodology: The study population was drawn from SUD patients who were positive to urine drug test. The serum CRP and IL-6 levels of 180 SUD patients (study group) were compared with the serum CRP and IL-6 levels of 180 apparently normal individuals who do not have history of substance abuse.

Results: The median and interquartile range of the ages of both the study group and the control group were 30 (23 – 40) and 33.5 (24-41) years respectively. The study population consist of 162 (90%) males and 18 (10%) females with median ages and range of 30.5 (15 – 72) and 26 (14 – 40) years respectively. A significant increase in IL-6 in SUD was observed (P = .0001) but no significant difference observed in CRP at 95% confidence interval (P = .73). It was observed that there was a strong positive relationship between IL-6 and CRP (r = 0.6646); P = .0001) in SUD patients.

Conclusion: IL-6 was significantly higher in people with SUD and as CRP levels increases IL-6 increases. This suggests that there is a level of inflammatory reaction in substance use disorder patients. Thus both serum CRP and IL-6 level can be considered as biomarkers of inflammation in patients with SUD.

ABBREVIATIONS

CRP   : C- reactive protein
IL   : Interleukin
SUD   : Substance use disorder
TNF   : Tumor Necrosis Factor
UNODC : United Nation Office on Drug and Crime.

1. INTRODUCTION

1.1 Background

Substance use disorder also known as drug use disorder is a condition characterized by the use of one or more substances which lead to a clinically significant impairment or distress [1] SUD comprises addiction and dependence but addiction characterizes the most severe form of the disorder [2]. SUD is characterized by overuse or reliance on a drug or substances resulting to consequences that are detrimental to the person's or other people's physical and mental health [3]. SUD was declared by United Nations Committee on Drugs and Crime as today's serious health and socio-economic issue worldwide [4]. According to the United Nations Office on Drugs and Crime (UNODC), it was estimated that 1 in 20 adults between the ages of 15 and 64 years, or a quarter of a billion people worldwide abused at least one drug in 2014 [4]. Substances most often associated with SUD include alcohol, caffeine, hallucinogens, inhalants, sedatives, hypnotics, anxiolytics, stimulants, tobacco and others [3]. It is a clear fact that certain substances and a range of illicit drugs above certain levels are harmful to human health. In SUD, more deaths, illnesses and disabilities have been reported than in any other preventable health condition [5]. In 2015 SUD resulted in 307,400 deaths, an estimate of 183,000 deaths was reported in 2012 up from 165,000 deaths in 1990 and these deaths occur in a population of those aged 15 to 65 years [4,6].

1.2 Inflammatory Markers

During inflammatory reactions, extra proteins are often released into the blood stream [7]. To detect this increase in proteins, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and plasma viscosity (PV) are commonly used. In this way they are usually referred to as markers of inflammation. Pro-inflammatory cytokines such as Interleukins 1 and 6 (IL-1, IL-6) and tumor necrosis factor alpha (TNF- α) induce the synthesis of some of the acute phase reactants which include CRP, fibrinogen and haptoglobin. [8]. Many results obtained from various researches on inflammation in substance use disorders are conflicting [8]. Different drugs have different chemical structures and therefore can affect the body in different ways, whether inflammation is one of the ways is ambiguous as evidence linking SUD with inflammation is contradictory and varies from one substance of abuse to another [9]. Substance abuse can have different inflammatory responses based on their route of administration [10]. Most SUD affect the functions of the Central Nervous System (CNS), the Autonomic Nervous System (ANS) and Gastro Intestinal Tract (GIT). However, they also affect other parts of the body including the respiratory and the cardiovascular systems [11] [12]. SUD has also been linked to neuroinflammation and alterations in neurogenesis [13]. The fact that the immune system does modify brain functions because of

substance abuse and the resultant participation of reward modulatory systems in psychiatric disorders shows the possibility of establishing a link between inflammation and addictive disorders [14].

Inflammatory markers, such as IL-6, tumor necrosis factor-α (TNF-α), and CRP, increase in response to infection and tissue damage and in active diseases states [15]. Inflammatory cytokines, including IL-6, interleukin-1β (IL-1β), and TNF-α, are cytokines involved in inflammation, which could be released quickly under pathological conditions, causing an inflammatory response in the central nervous system [16,17]. The use of biomarkers is advantageous because they are simple to administer and less time consuming [18]. Activating effects of substance abuse on the sympathetic nervous system make these substances a potential stress mediators of the immune system [19]. When psychologically stressed, the human body produces stress hormones like cortisol, which are able to trigger the release of interleukin-6 into the circulation [20].

1.3 Il-6 as an Inflammatory Marker

IL-6 is secreted by T cells and macrophages to stimulate immune response and is said to be the main cytokine involved in the induction of acute phase response proteins like CRP [21]. It is a newer serum inflammatory marker that has been successfully evaluated in multiple papers of periprosthetic infections. Literature has recognized the diagnostic utility of IL-6 and shows its superiority over traditional serum markers [22,23]. IL-6 has diverse immune functions that can stimulate or inhibit inflammatory responses depending on cellular context [24]. As a regulator of immune response and acute phase reactions, the concentration of IL-6 increases and returns to normal more quickly than CRP and ESR [25,26]. Interleukin-6 is the main proinflammatory cytokine activated in the innate immune process resulting in microglial activation [27]. Interleukin-6 has been found to be increased as an inflammatory marker in chronic heavy alcohol use [28]. IL-6 is also considered a myokine; a cytokine produced from muscle, which is elevated in response to muscle contraction [29].

1.4 Crp as an Inflammatory Marker

C-reactive protein is a systemic inflammation marker that is associated with chronic diseases [30,31]. CRP is an annular (ring-shaped) and an acute-phase protein of hepatic origin found in blood plasma, its levels increase in response to inflammation following interleukin-6 secretion by macrophages and T cells [32]. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells in order to activate the complement system via C1q [32,33]. CRP exhibits elevated expression during inflammatory conditions such as rheumatoid arthritis, some cardiovascular diseases, and infection [34]. In healthy adults, the normal concentrations of CRP vary between 0.8 mg/l to 3.0 mg/L [35]. When there is a stimulus, the CRP level can rise 10,000-fold from less than 0.5 mg/l to more than 500 mg/l. The rate of CRP production increases with inflammation, infection, trauma, necrosis, malignancy, and allergic reaction [35]. Changes in serum CRP concentration occur more quickly than ESR and therefore CRP may be a better reflection of current inflammation. Unlike the ESR, CRP is a fairly stable serum protein whose measurement is not time-sensitive and is not affected by other serum components [36]. The magnitude of inflammation directly relates to the concentration of CRP. Levels less than 0.2 mg/dl are considered normal, while those greater than 1.0 mg/dL are suggestive of inflammation and/or infection. More recently, the use of high sensitivity CRP has been utilized. This test may better quantify lower levels of inflammation and has been important in evaluating cardiac disease and other inflammatory states [36,37]. Like IL-6, increase in CRP is also associated with mortality risk [38]. The potential significance of IL-6 and CRP among tobacco smokers was linked to the growth and progression of many malignancies [39,40,41].

CRP levels were seen to be high in the presence of SUD such as nicotine, alcohol, and cannabis use disorder. In prospective analyses, higher CRP levels predicted cannabis use and nicotine dependence, and nicotine use predicted higher CRP levels [8]. Elevated serum CRP levels are associated with endothelial dysfunction, coronary artery, calcification and cardiac diastolic dysfunction in chronic cocaine users [42]. The inter-relationship of CRP and substance abuse has implications for the later health risks associated with early SUD [8]. Cigarette smoking has also been associated with increases in CRP [15]. Many researchers later discovered that the increased CRP levels are a secondary effect of cigarette smoking and it only reflects tissue injury [43].

The information available on inflammatory markers in substance use disorder are very scanty and showed many variations in results thereby prompting the need for continuous research in order to adapt possible policy in the treatment and prevention of the comorbidity. However there are few reliable researches on substance use disorder and inflammatory reaction which in Nigeria is extremely scanty; this provided the impetus for conducting this study.

1.5 Objectives

The objective is to determine the relationship between the serum levels of inflammatory markers (IL-6 and CRP) and substance use disorder (SUD) patients.

2. METHODOLOGY

2.1 Study Area

This study was conducted at Federal Neuro-psychiatric Hospital (FNPH) Kaduna, North-western Nigeria between August 2018 and August 2019. The hospital and its Medical Laboratory serve as a referral center for patient with psychiatric disorders from all the North West states and the Federal Capital Territory, Abuja.

2.2 Study Design

This is a cross sectional comparative study. The participants and the controls comprised 360 persons sent to the laboratory for drug abuse tests.

2.3 Study Population

The study population was drawn from substance or drug abusers who are positive to urine drug test and have been taking the substance for at least three months. Substance abuse was defined as use of any potentially addictive substance, in the past 3 months as defined by the fifth (5th) edition of the Diagnostic and Statistical Manual of the American Psychiatric Association [3]. The Urine sample of all the 360 participants were collected and using drug abuse panel test kit, the drug abuse status of participants was ascertained.

2.4 Minimum Sample Size Determination

The minimum sample size was calculated using the WHO Health Survey Statistics formula: N =

1.96^2 x P (1-P)/d^2 [44]. *Where:* N = Minimum sample size P = Prevalence rate d = Precision.

Based on a previous study of drug abuse in Nigeria by United Nation on drug and Crime (UNODC) in 2017, the incident rate of drug abuse for the North West of Nigeria was said to be 12%. [45] Therefore, prevalence rate (p) was 12% (0.12) and having a confidence interval of 95%, the precision (**d**) was 0.05 or 5%. Using the formula above, the minimum sample size was calculated to be 162. However, 180 people positive to at least one substance of abuse were studied alongside with 180 apparently normal control individuals who do not have history of substance abuse.

2.5 Inclusion Criteria for Study Group

All persons positive to one or more substance of abuse and have been taking the substance(s) for at least three months, Participants negative to Hepatitis B and C, HIV and Pregnancy test (if female), and Participants with normal full blood count and BMI.

2.6 Inclusion Criteria for Control Group

Persons with no history of substance use and negative urine test to substance of abuse, Persons who are sero-negative to hepatitis B and C and HIV, Female participants with negative pregnancy test results, Participants with normal full blood counts and Participants with normal body mass index (BMI).

2.7 Exclusion Criteria for Study and Control Group

Sick individuals and those on any medication, those who declined consent., women who were obviously pregnant and those with positive pregnancy test, and substance abusers who have not reached three months when they started abusing drugs, individuals with abnormal full blood counts and BMI (< 18 and ≥ 25) and those Positive to HIV screening, Hepatitis B, and C test.

2.8 Sample Collection

Participants were allowed to relax for at least 1 hour prior to sample collection. Six (6) milliliters (mls) of blood sample was collected from each study participant and control into plain tubes (4 mls) and EDTA (2 mls). The blood in plain

tubes were allowed to clot, and the dislodged blood was centrifuged for 5 minute at 4000 revolution per minutes and the serum was separated into cryovials, with aprotinin added and was stored at -80°C till analysis for interleukin-6 and C-reactive protein. Urine sample was collected into sterile universal container.

2.9 Sample Analyses

These describe the analyses of the samples of both the study and the controls.

2.9.1 Materials needed

Microplate reader, Precision, pipettes, disposable tips, Adjustable 10 ml – 1000 ml pipettes for reagent preparation, One hundred milliliter and 1 litre graduated cylinders, Absorbent, paper, 37°C incubator, Distilled or deionized water, Eppendorf Tubes for diluting samples.

After all kit components and samples have been brought to room temperature (18-25°C) the following procedure was below.

2.9.2 Urine Drug of Abuse (DOA)

DOA test was done using EUGENE DOA multiple rapid diagnostic test panel by EUGENE Biotech Co. Ltd Shanghai China. The test kit, the urine sample and the control were allowed to reach room temperature prior to testing. The test is based on highly specific antigen and antibody immunochemical reaction. Absence of the colored band on the test region indicates a positive result, the presence indicates Negative and the absence of the control band indicates invalid.

2.9.3 Interleukin-6 assay

2.9.3.1 Test principle

The micro titer plate provided in the kits (WKEA MED SUPPLIES CORP) has been pre-coated with purified antibody specific to IL-6 to make a solid phase. When Standards and samples were added to the appropriate microtiter plate wells, the interleukin-6 in the sample combine with enzyme labeled goat anti human and became antibody-antigen-enzyme-antibody complex which after washing completely and substrate is added, turns blue color with the addition of HRP which catalyzed it. The reaction is terminated by the addition of a sulphuric acid solution and the color change to yellow which is measured spectrophotometrically at a wavelength of 450 nm. The concentration of interleukin-6 in the sample is then determined by comparing the O.D of the samples to the standard curve.

2.9.3.2 Procedure for interleukin-6

The procedure followed was as outlined by the kit manufacturer's instructions. Five wells for standards and one well for blank were prepared out of the 96 wells. Fifty microliter (ul) of the diluted standards was added into the wells for standards. Forty microliter of sample diluent was added to all the wells for samples and 10 ul of all samples was added to the same wells to make a one in five dilution for each sample. While for blank well the same process was followed except that no sample nor enzyme conjugated was added. The plates were mixed gently by rocking for fifteen seconds. The plate was closed with plate membrane and incubated for 30 minute at 37°C. The plate was decanted into a sink and each well was filled with wash solution and was again decanted into the sink. This washing procedure was done for five times. After the washing the plate was inverted and blotted dry.

Fifty microliter of enzyme conjugate reagent was added to each well except the blank well. The plate was then incubated for 30 minutes at 37°C. After this another procedure of washing was done 5 times. The plate was inverted and blotted dry. Fifty microliter of substrate A and 50ul of substrate B was added to each well, and it was incubated for another 15 minutes at 37°C, after the incubation, the reaction was stopped by the addition of Stop solution. The plate was then shaken gently and read directly at 450 nm within 15 minutes by a microplate reader (BIO RAD PR 5100).

2.9.4 C reactive protein assay

2.9.4.1 Test principle

CRP ELISA Kit (Accubind) was used. It is an immunoenzymometric assay requiring high affinity and specificity of antibody with different and distinct epitope recognition. It requires immobilization through the interaction of septavidin and biotinylated monoclonal anti-CRP antibody. Upon mixing with a serum containing the native antigen a soluble sandwich complex is formed. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decanting or aspiration. The

enzyme activity in the antibody bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown sample can be ascertained.

2.9.4.2 Procedure for CRP assay

The procedure was as outlined in the kit manufacturer's instruction. Serum diluent was diluted to 200 mls in a suitable container with distilled water. Two milliliter of diluent was dispensed into all the eppendorf tubes for the number of samples to run and into these, 10ul of each sample was added and it was mixed thoroughly and stored at 2 – 8°C for 48 hours.

All kit components and samples were brought to room temperature (18-25°C). Wash solution was diluted to 1000mls with distilled water in a suitable container. Solution A was poured into solution B. 6 wells for standard and sample were determined from 96 wells. Twenty five microliter of the appropriate of the serum reference and samples were dispensed into assigned wells. One hundred microliter of the CRP enzyme reagent was added to each well with multi channeled pipette. The microplate was swirled gently for 20 to 30 seconds and incubated for 15 minutes at room temperature. After incubation the content was discarded by decantation, it was tapped and blotted unto absorbent paper.

Wash buffer of 350ul was added unto each wells, it was decanted and blotted unto absorbent. This washing was done 3 times. Working substrate solution of 100ul was then added to all wells and the plate was again incubated for 15 minutes at room temperature. After the incubation 50ul of Stop solution was added to each well, and the plate was mixed gently for 15 – 20 seconds. The absorbance of the resultant yellow solution was read within 30 minutes at 450nm in a microplate reader (BIO RAD PR 5100). A reference curve was developed to ascertain the concentration of CRP in the samples.

2.10 Statistical Analysis

Microsoft Excel was used to collate the data and Graph Pad prism (version 6) was used to analyze the data. Spearman correlation coefficient was used to measure continuous variables and Mann-Witney U test was used to compare continuous variables in the two groups and to determine the relationship between

Substance use disorder and control group. The results being non-parametric tests were expressed as the median and interquartile range. A *p* value less than 0.05 was considered as statistically significant.

3. RESULTS

3.1 Sociodemographic Characteristic

The study population consist of 162 (90%) males and 18 (10%) females with median ages of 30.5 (15 – 72) years and 26 (14 – 40) respectively. The median (interquartile range) for the age of the study and control group were 30 (23 – 40) and 33.5 (24 – 41) years respectively. No statistically significant difference was detected in age with p-value 0.81. The age of the study group ranged from 14 to 72 years. The age range 24 to 33 years had the highest participants with 58 (32.2%) substance abusers while age range 64 to 73 had the least number of participants with 4 (2.2%) substance abusers which could be as a results of low rate of elderly people coming for drug abuse test. Due to low rate of female individual coming for drug abuse test only 18 (10%) were qualified for inclusion into the study and 18 (10%) were also included into the control group. Seventy nine (43.9%) of the substance abusers were students followed by farmers with 27 (15.0%) participants and the occupation that had the least participation among those on substance abuse were the Non-Governmental employee with 4 (2.2%) substance abusers. (Table 1). The frequency of the tribes of those abusing substances in this study was also deduced with the Hausa having the highest participation with 77 (42.8%) substance abusers probably because of the region from which the study was done while the Igbos had the least participation with 11 (6.1%) substance abusers. Most of the substance abusers in this study were married with 101 (56.1) married substance abusers while 5 (2.8%) were widows or widowers. (Table 1).

3.2 Pattern of Substance Abuse

The 180 participants who were positive to at least one drug of abuse test comprised 75 persons (28.7%) who abuse cannabis, 79 people (30.3%) who abuse cigarettes, 33 (12.6%) people who abuse benzodiazepine, 36 people (13.8%) who abuse tramadol, 7 people (2.6%) who abuse codeine, 2 (0.8%) people who abuse amphetamine, 1 person (0.4%) who abuses barbiturate, 12 people (4.6%) who abuse pentazocin, 8 (3.1%) people who abuse

morphine, 3 (1.2%) people who abuse alcohol, 2 (0.8%) people who abuse phencyclidine and 3 (1.2%) people who abuse cocaine irrespective of whether they combined the substance with other substances or not (multiple frequency: M.F) as seen in the Table 2. Nicotine which is in the form of cigarette is the most commonly abused substance followed by cannabis, benzodiazepine and tramadol while Barbiturate was the least commonly abused substance in this study.

According to the results, most of the people on cigarettes are males between 15 and 72 years while the few female smokers are between 17 and 40 years. The results also indicated that males who abuse substances cut across all age groups but females above 40 years were not observed abusing substances in this research. Females were not also observed abusing pentazocin, cocaine, amphetamine, phencyclidine and barbiturate.

**Table 1. Sociodemographic characteristics of substance abusers**

Age range (Median (IQR) = 30 (23-40))	Male	Female	Total (%)
14-23	39 (21.7)	6 (3.33)	45 (25.0)
24-33	52 (28.9)	6 (3.33)	58 (32.2)
34-43	36 (20.0)	6 (3.33)	42 (23.3)
44-53	19 (10.6)	0 (0.0)	19 (10.6)
54-63	12 (6.7)	0 (0.0)	12 (6.7)
64-73	4 (2.2)	0 (0.0)	4 (2.2)
Total	162 (90)	18 (10.0)	180(100)
Occupation			
Student	71 (39.4)	8 (4.4)	79 (43.9)
Unemployed	19 (10.6)	5 (2.8)	24 (13.3)
Artisan	8 (4.4)	0 (0.0)	8 (4.4)
Civil servant	9 (5.0)	2 (1.1)	11 (6.1)
Non-Government employee	2 (1.1)	2 (1.1)	4 (2.2)
Business m/w	8 (4.4)	1 (0.6)	9 (5.0)
Farmer	27 (15.0)	0 (0.0)	27 (15.0)
Retiree	3 (1.7)	0 (0.0)	3 (1.7)
Commercial Driver	19 (10.6)	0 (0.0)	19 (10.6)
Commercial motorcycle rider	16 (8.9)	0 (0.0)	16 (8.9)
Tribes			
Hausa	69 (38.3)	8 (4.4)	77 (42.8)
Yoruba	18 (10.0)	1 (0.6)	19 (10.6)
Igbos	11 (6.1)	0 (0.0)	11 (6.1)
Fulani	9 (5.0)	3 (1.7)	12 (6.7)
Others	45 (25.0)	6 (3.3)	51 (28.3)
Total	162(90)	18(10.0)	180 100)
Marital status			
Single	66 (36.7)	8 (4.4)	74 (41.1)
Married	91 (50.6)	10 (5.6)	101(56.1)
Widow	5 (2.8)	0 (0.0)	5 (2.8)
Total	162 (90)	18 (10.0)	180 (100)
Educational attainment			
Primary	31 (17.2)	5 (2.8)	36 (20.0)
Secondary	66 (36.7)	6 (3.3)	72 (40.0)
1st Degree	50 (27.8)	7 (3.9)	57 (31.7)
2nd Degree	3 (1.7)	0 (0.0)	3 (1.7)
Uneducated	12 (6.7)	0 (0.0)	12 (6.7)
Total	162 (90)	18 (10.0)	180 (100)
Religion			
Muslim	106 (58.9)	11 (6.1)	117 (65.0)
Christian	56 (31.1)	7 (3.9)	63 (35.0)
Total	162 (90)	18 (10.0)	180 (100)

Table 2. Pattern of substance abuse

		Study group		Sex		
		Participants		**Male**		**Female**
		(180, 100%) Median Age (Range) 30 (15 – 72)		(162, 90%) Median Age (Range) 30.5 (15–72)		(18, 10%) Median Age (Range) 26 (14 – 40)
Substances						
Nicotine	79	31 (16-72)	68	32 (16-72)	11	26(17-40)
Cannabis	75	31 (15-71)	67	33(15-71)	8	22(15-38)
Tramadol	36	29.5(18-70)	33	30(18-70)	3	25(15-38)
Benzodiazepine	33	32(18-55)	30	32(20-55)	3	26(18-35)
Pentasocine	12	34.5(21-46)	12	34.5(21-46)	0	0
Morphine	8	34 (14-63)	7	36(20-63)	1	14
Codeine	7	32(24-35)	5	32(24-34)	2	30.5(26-35)
Alcohol	3	22(20-26)	2	24(22-26)	1	20
Cocaine	3	25(20-28)	3	25(20-28)	0	0
Amphetamine	2	35.5(33-38)	2	35.5(33-38	0	0
Phencyclidine	2	35(27-43)	2	35(27-43)	0	0
Barbiturate	1	18	1	18	0	0

Table 3. Comparison between biomarkers of inflammation in SUD and control group

Biomarker	Median (IQR)		P-value (#)
	SUD (180,100%)	**Healthy control (180,100%)**	
CRP (µg/mL)	2.9 (0.8 – 11.5)	4.2 (2.3 – 5.4)	0.73
IL-6 (ng/L)	13.5 (5.9 – 20.1)	4.1 (2.8 – 5.4)	0.0001*
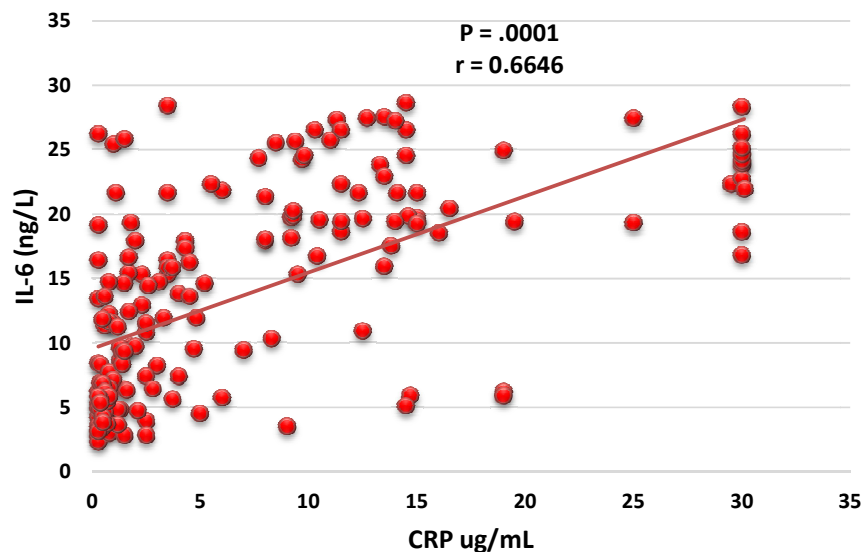
Fig. 1. Scatter plot showing relationship between interleukin 6 (IL-6) and C-reactive protein

3.3 Levels of CRP and IL-6 in Study Population and Control

The values of CRP and IL-6 of patients with SUD and that of the controls were compared using Mann-Whitney U test as shown in Table 3. A significant increase in Interleukin 6 in substance use disorder was observed (p-value = 0.0001) while no significant increase was observed in the CRP. This means that the interleukin 6 levels of those abusing substances are higher than those who are not abusing substances.

3.4 Relationship between Interleukin 6 and C - reactive Protein

It was observed that there was a strong positive relationship between interleukin 6 and C-reactive protein in substance use disorder (r = 0.6646), which is significant (p = 0.0001). The scatter graph is shown in Fig. 1.

4. DISCUSSION

This study involved 180 SUD patients between 14 and 72 years which coincide with the report of the national drug survey by UNODC in collaboration with the National Bureau of Statistics (NBS) and Centre for Researcher and Information on Substance Abuse (CRISA) which shows that those involved in substance abuse in Nigeria are between 15 and 64 years of age [45]. Each of these individuals was abusing at least one drug as at the time of sample collection. The median (interquartile range) of the age of the study group was 30 (23-40) years. The drug abusers were divided into age groups with age group 24 to 33 years having the highest occurrence of substance use. This agrees with the survey lead by NBS and CRISA which reported that the highest level of substance use disorder was found among people between the ages of 25 to 39 [45].

Nicotine which is in the form of cigarette is the most commonly abused substance in this study with 33.3% (79 people) abusing it, probably because of the relatively easy way of obtaining it and not termed as illegal in Nigeria followed by cannabis with 28.7% (75 people). Tobacco smoking is the most popular substance of abuse and is said to be the most important cause of preventable and premature death globally. The WHO estimated that tobacco kills nearly seven million people annually and 100 million death were recorded over the course of the 20th century. More than six million of those deaths were the results of direct tobacco use [46]. This result was different from that of UNODC survey which placed cannabis as the most abused substance in Nigeria although in their survey cigarette and tobacco were excluded [45]. Cannabis was said to be the most commonly used drug in Nigeria with 10.8% (10.6 million) of the adult population reporting use in the past five years [45], these reasons probably account for both substances coming up as the most occurred substance of abuse in this research. Females were not also observed abusing pentazocin,

cocaine, amphetamine, phencyclidine and barbiturate although these substances were generally low in frequency in this research, probably because these substances were not available over the counter and also some of them are termed controlled drugs and therefore they are not easily obtained. The ratio of males to females in the intake of substance of abuse was 9 to 1 in this study. This was based on the fact that most people coming for drug abuse test were male while the rate of females coming for drug abuse test was generally low.

Students, mostly undergraduates were seen to have the highest participation with 79 (43.9%) substance abusers. This agrees with many researchers who reported high levels of substance abuse among students in some parts of Nigeria such as Osun state [47] Ighoora [48] Ibadan [49] Oshogbo [50] Jos [51]. One of the contributors to the increase in students' population among substance abusers in this study could be the facts that there are many secondary and tertiary institutions in Kaduna where this study was conducted. The stress of education as well as peer pressure could also drive this observation Next to students were the farmers followed by the unemployed.

The tribe with the highest participation was the Hausas. This was probably because this study was done in the North Western part of Nigeria dominated by Hausas. The UNODC also reported high prevalence of 12% of substance use in North West of Nigeria which was predominantly Hausas. The married were seen to have the highest frequency of substance abuser in this research. This is however contrary to some researchers who reported highest frequency of substance abuse in those who were not married [52,53,54]. Those with secondary school certificate as the highest certificate they have were seen to have the highest participation, and most of them were undergraduate. Undergraduates have always been implicated in researches that involve substance abuse [55]. In Nigeria it is a recognizable phenomenon according to Makanjuola et al, in 2007 who reported high prevalence of substance abuse among under graduates [56]. The Muslim were seen to have higher participation than the Christian probably because of the region where the study was done which is North Western Nigeria dominated by the Muslims. This is not in agreement with Ajibolu et al in 2018 who reported that Christians take higher frequency of

those abusing substances in Oyo State, South West Nigeria [57].

Another notable observation is the significant increase in serum IL-6 in SUD when compared with non-SUD. This result is in concurrence with that of Fernanda et al. (2015), where serum levels of IL-6 were increased in cocaine users [58]. A study performed by Ersche et al. in 2014 also found increased salivary levels of IL-6 in cocaine-dependent men when compared to the control group who had no personal or family history of substance use disorders [59].

In this study, a significant positive relationship was observed between serum interleukin 6 and C-reactive protein in the study group. No research was seen as at the time of this research which correlates CRP and IL-6 among SUD patients, however this research agrees with McArdie et al. in 2004 in United Kingdom who also found a significant positive relationship between IL-6 and CRP in prostrate disease [60]. Also Il'yasoba et al in 2008 found a positive correlation between IL-6 and CRP in patients with high risk of cardiovascular disease [61]. Although there were very few research which do not find any correlation between IL-6 and CRP like Czarkowaska-paczek et al in 2005 in healthy male athlete probably as a result of the activity of the athletes [21]. Many researchers have shown that interleukin 6 is one of the pro-inflammatory cytokines that stimulate the production of C-reactive protein [62].

Literature shows that most researchers only depend on questionnaire to get data on substance use disorder unlike in this study in which the presence of these substances were confirmed by drug abuse test before including them into the research. This could be the reason for the differences in the results obtained.

The constraint was that this study could not evaluate the concentration or level of the substances in the participants. This was also a hospital based study which could also contribute to the differences between the results obtained and that of other researchers.

5. CONCLUSION

In conclusion serum interleukin-6 is higher in people with SUD and it was also found out that IL-6 increases as C-reactive protein increases in patients with SUD and since both IL-6 and CRP are markers of inflammation, this therefore mean that inflammatory reactions occur with substance use disorder.

6. LIMITATION

The constraint was that this study could not evaluate the concentration or level of the substances in the participants. This was also a hospital based study which could also contribute to the differences between the results obtained and that of other researchers.

CONSENT AND ETHICAL APPROVAL

Ethical clearance was obtained from Federal Neuropsychiatric Hospital Kaduna. A written informed consent from the patients and the relatives of the patients was sought for and obtained prior to the commencement of samples collection.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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