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Haematological and Haemostatic Changes Associated with Major Depressive Disorder among Nigerians.

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Abstract

Background: A link between major depressive disorder (MDD) and haematological as well as coagulation disorders has been postulated. This study aims to evaluate haematological and haemostatic changes among Nigerians with major depressive disorder

Methods: Two hundred volunteers consisting of an equal number of individuals diagnosed with major depressive disorder (MDD) based on DMS-IV criteria and apparently healthy control participated in this study. The blood sample was collected into tri-sodium citrate K2EDTA bottles respectively and was evaluated for some haemostatic parameters , using ELISA, Clauss, Quick's One Stage, Proctor and Rapaport's methods.

Results: The mean WBC, hemoglobin and differential lymphocyte were significantly higher among MDD total volunteers ($p < 0.001$). The red cell indices and platelet count were lower among MDD ($p < 0.001$). Also the prothrombin time (PT), fibrinogen, protein-C and erythrocytes sedimentation rate (ESR) were all raised ($p < 0.001$) among volunteers with MDD. Positive associations existed between MCV and RBC ($r: 0.364$; $p < 0.001$), PT and APTT ($r: 0.319$ $p < 0.001$), APTT and fibrinogen ($r: 0.239$, $p = 0.017$) as well as PT and fibrinogen ($r: 0.275$ $p = 0.006$) at 95% confidence interval.

Conclusion: Changes in total leucocytes count, lymphocytes values and haemostatic parameters among volunteers with depression may impacts deleterious effects on the immune response as well as haemostatic homeostasis, while decreased red cell indices may suggest occult nutritional anaemia.

Keywords: Depression, haematological parameters, Red cell indices, Protein C, D-Dimer, Fibrinogen.

1.0 INTRODUCTION

Major depressive disorder (MDD) has been described as a medical condition that primarily affects an individual's mood as well as behaviour [1]. Available data has suggested that affected individuals present with a myriad of features which constitute their dispositions to life in general [2, 3].

It has been suggested that major depressive disorder may be a risk factor for complications and death in individuals with ischaemic heart disease [IHD] [4, 5]. This disorder could also present in different forms [6].

Furthermore, some haematological disorders may be seen in individuals with depressive disorder. While there are few data that demonstrated the negative impact of depression on coagulation derangements [7-9], there is a paucity of similar studies among Nigerians with depressive illness. Thus this study is set to further build on the previous studies with particular reference to Nigerian depressive disorders volunteers.

It appears that the relationship between Major depressive disorder and haematological changes has not been extensively studied. However, there was a previous study that evaluated some hematology indices in manic depressive disorder [10].

The previous study has opined that major depressive disorder and anxiety tend to be closely related, as the genes coding for the symptoms of MDD possibly influences the symptoms of anxiety [11].

It has been shown in some previous studies that most Major depressive disorder and anxiety disorders might manifest early in life in affected individuals [11-13]; while available data suggested that MDD that manifests later in adulthood might have resulted from specific and different etiological factors [14-17].

2.0 METHODOLOGY

2.1 Study Design and Location

This is a cross sectional study carried out over a period of six months. The study was carried out at the Federal Neuro-psychiatric, Yaba, Lagos State, Nigeria.

2.2 Study Population

Participants for this study were randomly recruited from the outpatient of the Federal Neuro-psychiatric Hospital,

Yaba, Lagos State. A total of two hundred volunteers, participated in this study. The test participants (case) consist of 100 MDD [6] patients while the control group consists of 100 age-matched apparently healthy volunteers. MDD patients recruited was based on DMS IV criteria. Participants were randomly selected and prevalence of 3.1% (lifetime) and 1.1% (12-months) based on population-based world mental health survey initiative in Nigeria [18]. Participants recruited were those who have been diagnosed of MDD and have been receiving treatment for at least six months.

Information on socio-economic and demographic status of participants was taken through questionnaire design for the study.

2.3 Ethical Considerations, Inclusion and Exclusion Criteria

Approval for this study was obtained from the ethics and research committee of the Federal Neuro-Psychiatric Hospital, Yaba Lagos Nigeria [FNPHY/ERC/14/102]. This study was conducted in the same hospital. Thus participants consent was sought and obtained before they were enrolled for this study.

Participants (male and female) with depression aged 30 to 70 as well as apparently healthy age matched individuals which served as control were included.

Those excluded are individuals below 30 years of age and above 70 years. Also individuals with any other form of ailment(s) apart from depression were excluded from this study.

2.4 Laboratory Methods

Collection of Blood Samples: Blood sample was collected from each participant into sodium citrate and K2EDTA bottles respectively. The samples were used for the haematological studies respectively.

The Erythrocyte sedimentation rate was determined using Westergren method [19]. This method involve the usin of Dispette 2 Citrate (GS-1650) manufactured by Guest Scientific AG. Activated Partial Thromboplastin Time was determined by Proctor and Rapaport's modification method [20] using commercially prepared Kaolin Platelet Substitute Mixture manufactured by Diagnostic Reagents Ltd. Fibrinogen Estimation was carried out based on Clauss method [21] using Fibrinogen Determination Kit manufactured by Diagnostic Reagents Ltd. Prothrombin time test was

determined using Quick's One Stage method [22]. Protein C and D-dimer were determined using ELISA method [23]. Full Blood Count was determined based on Coulter's principle [24-26].

2.5 Statistical/Data Analysis

Data were analyzed using SPSS version 20 statistical packages. Descriptive statistics of mean $\pm$ SEM was used to examine the data. Independent student's t- test was used to compare groups. Pearson correlation analysis was conducted to evaluate the degree of association of the parameters studied. The probability value of $p < 0.05$ was considered significant for this study.

3.0 RESULTS

The mean age of the participants were 47.0 ± 1.23 and 48.1 ± 0.97 years for test and control respectively. Age group 30-40 had more volunteers with depression (39%) of all the age ranges while age group 61-70 had the least (17%). In the overall, 53% and 47% represented the percentage of male and female volunteers respectively with depression in this study.

The mean white blood cell and red blood cell counts were significantly ($P < 0.05$) higher among MDD participants. On the other hand mean haemoglobin, platelet and red cell indices were lower ($p < 0.05$) in MDD patients

3.1 Haemostatic Parameters

The prothrombin time test, protein C, Fibrinogen and erythrocytes sedimentation rate in MDD were higher ($p < 0.05$) than control.

3.2 Pearson Correlation Studies

The degree of association between continuous variables were evaluated. There was a positive association ($p < 0.05$) between MCV and RBC, Monocytes and platelets and between PT and APTT, PT and Fibrinogen among patients with major depressive disorder.

3.0 DISCUSSION

Depressive disorders are significant medical disorders which are associated with psychiatric symptoms [27]. It is characterized by disruption in mood as well as the behaviour of the affected individuals [1]. In this study, we evaluated some haematological, and hemostatic parameters

among volunteers who have been diagnosed with the major depressive disorder using defined standard procedures [6]. The mean age (table 1) of the test volunteers was not different from the control. This was expected as the study design was to study age matched case and control participants with a view to nolyfy any possible impact of diversity in age of the study population. A previous study, by Sebastian [28] suggested that ageing may be a possible risk factor for depression. While some changes are normal with ageing, a study has shown that depression is not a function of ageing. It is instructive to note that ages 31-40 had the highest number of volunteers with the depressive disease (figure 1). This observation may suggest the possible increased burden ranging from economic, societal, family and marital burden on the younger age generations in Nigeria. It is also good to note that 53 and 47 test participants representing 53% and 47% were male and female respectively (figure 2). This observation suggests that both sexes are affected by depression among Nigerian population. However, in this study, the male participants were slightly more affected than the female (figure 2). Previous studies have suggested that in most cases, depressive illness and anxiety disorder occur early in life [11-13]. Available data have also opined that genetic and environmental factors may play key roles in the development of depressive and anxiety illnesses [11-13]. In this study, however, it was observed that the incidence of depression appears to be higher among young adults age 30 to 40; thus agreeing with the previous studies by Kendler *et al.*, [11], Kendler *et al.*, [12], and Kendler *et al.*, [13]. It thus appears that ageing may not be a major risk factor for depressive illness.

We observed that the test group had a significantly higher total white blood cell count and lymphocyte differential counts when compared with the control. The white blood cells play a cardinal role in human's immunological response. An increase in total white blood cell count, as well as lymphocytes, differential count among individuals with major depressive disorder when compared to the apparently healthy control could suggest that depression may affect the immunological response in affected individuals. This is because the white blood cells particularly the lymphocytes cells are critical in cell mediated immune responses in humans. Thus whatever affects these cells may indirectly impact on the immune response of an individuals. Available data on the effect of depression on immunological response and immunologi-

Figure 1. showing the age distribution of the participants

Table 1. Haematological Descriptive Statistics for both Test and Control Group .

Variables n=100	Test group Mean±SEM	Control group Mean±SEM	t-test	p value
Age(yr)	47.0±1.23	48.1±0.97	0.539	0.446
WBC	5.5±0.15	4.6±0.10	4.583	<0.05*
($\times 10^9/L$)				
RBC	4.5±0.05	4.1±0.07	4.433	<0.05*
($\times 10^{12}/L$)				
HB(g/dl)	11.9±0.13	13.78±2.34	-5.397	<0.05*
PCV(L/L)	0.4±0.01	0.4±0.01	0.306	0.760
PLT($\times 10^9/L$)	163.3±4.78	205.0±7.22	-4.378	<0.05*
MCH	25.8±0.25	33.8±0.60	-12.137	<0.05*
(pg)				
MCV(fl)	82.5±5.58	90.1±1.30	-5.294	<0.05*
MCHC	312.0±0.15	370.9±2.14	-1.5791	<0.05*
(g/L)				
NEU(%)	48.4±1.21	48.3±0.10	0.058	0.954
LYM	48.2±1.15	42.6±0.94	3.543	<0.05*
(%)				
MON	2.6±0.33	7.1±0.15	-11.699	<0.05*
(%)				
EOS(%)	1.0±0.17	1.9±0.28	-2.726	<0.05*
BASO	0.0±0.00	0.1±0.03	-2.935	0.004*
(%)				

WBC – White Blood Cell, RBC – Red Blood Cell, HGB –Haemoglobin Concentration, HCT – Haematocrit, MCV - Mean Cell Volume, MCH - Mean Corpuscular Haemoglobin, PLT – Platelet, MCHC – Mean Corpuscular Haemoglobin Concentration, NEUT- Neutrophils, LYM-Lymphocytes, MON-Monocyte, EOS-Eosinophils, BAS-Basophils, *significant p value (p<0.05).

Figure 2. Showing the Distribution of test and Control

Table 2. Evaluation of degree of association among the parameters evaluated.

Variable	Correlation Coefficient (r)	P (value)
Red blood cell and Packed Cell Volume	0.825	<0.001*
Hemoglobin and Packed Cell Volume	0.980	< 0.001*
Mean Cell Volume and Red Blood Cells	0.364	<0.001*
Neutrophil and Lymphocytes	-0.944	<0.001*
Monocyte and Neutrophil	-0.379	<0.001*
Monocyte and Platelet	0.229	0.022*
Prothrombin and APTT	0.319	< 0.001*
PT and FIB	0.275	0.006*
APTT and FIB	0.239	0.017*
Protein-C and D-dimer	0.045	0.656
Protein-C and ESR	0.103	0.309

PCV – Packed Cell volume, RBC – Red Blood Cell, HGB – Haemoglobin Concentration, MCV - Mean Cell Volume, NEUT- Neutrophils, LYM- Lymphocytes, MON- Monocyte, EOS- Eosinophils, BAS- Basophils, PT- Prothrombin Time, APTT- Activated Partial Thromboplastin Time, FIB- Fibrinogen Concentration, ESR- Erythrocyte Sedimentation Rate

cal dysfunction has been inconsistent [29]. Some of the possible reasons for these inconsistent observations might be due to the etiological variability of depression [29].

Figure 3a and 3b. Coagulation and Inhibitor of Coagulation Profile among the Participants.

PT-Prothrombin Time, APTT-Activated Partial Thromboplastin Time, FIB-Fibrinogen Concentration, ESR-Erythrocyte Sedimentation Rate

However, a previous study showed that alteration in mood control aspect of nervous system possibly impacts deleterious effects on the immunological integrity of the affected individuals [30]. This by extension may impact negatively on the behaviour of the affected individuals [30]. Thus a significant increase in the lymphocytes differential count may suggest that depressive disorders could modulate the immunological responses of the affected persons.

In addition to this, the mean Prothrombin time and Fibrinogen were significantly higher (Figure 3a) among volunteers with depressive illness when compared with the control group, whereas the mean APTT was observed to be lower ($p < 0.05$) among the MDD volunteers. A prolonged prothrombin time in the presence of normal APTT may suggest a possible factor VII deficiency [31] among volunteers with depressive illness. This study agrees with the previous study by Canan *et al.*, [7], who observed a link between depression and some haematological disorders. It could also probably mean that there may be a possible relationship between deficiency of factor VII and increase in some natural inhibitors of coagulation such as protein C. The outcome of this study possibly corroborate a previous study by Geiser *et al.*, [9] who reported that abnormal coagulation proteins activations, particularly in depressive patients on medication, exist, leading to abnormal plasma fibrinogen and fibrinolytic activities; however, the mechanism for this abnormal activation is not fully elucidated. It is worthy of note that the volunteers with depressive illness demonstrated a significant increase in the mean fibrinogen level (Figure 3a). This observation in particular seems to be in consonance with the previous observations from the studies by Geiser *et*

al., [9], Panagiotakes *et al.*, [32] and Wiun-Anderson *et al.*, [33] demonstrated an increase in plasma fibrinogen among individuals with depression. This could also be associated with some degree of inflammation as demonstrated by an increase in the mean erythrocytes sedimentation rate among individuals with major depressive disorder (Figure 3a).

In this study, we observed an increase ($p < 0.05$) in the mean Protein-C concentration among volunteers with depressive illness. While protein C activation from its inactive zymogen to active protease involves cleavage by thrombin at the Arg169-Leu170 peptide bond, this cleavage is accelerated by thrombomodulin and endothelial protein C receptor (EPCR) [34-37]. However, the mechanism of activated protein C (APC'S) direct anticoagulant activity involve factors V and VIII which are homologous coagulation cofactors that circulate in inactive molecules; these are converted to active cofactors by limited proteolysis. Irreversible proteolytic inactivation of factors Va and VIIIa by APC results in its anticoagulant activities. An increase mean value of protein C in this study, however, did not lead to an increase in the mean APTT level of depressive illness volunteers as one would have thought. The reason for this observation is sparse; however, the effect of drugs and other depression factors may play a role. On the other hand, the D-Dimer value among test and control groups did not vary significantly (Figure 3b).

An increase in the mean erythrocyte sedimentation rate as observed among the depressive illness group suggests that this disorder may have a link with inflammation. Previous studies have however suggested a link between inflammation and depressive illness. Available data suggested that depression is associated with a higher concentration of some inflammatory cytokines [38-40]. Krishnasdas and Cavanagh [39] however reported that in some predisposed individuals, stress could induce proinflammatory cytokine release which in-turn stimulates the release of extrahepatic enzyme Indoleamine 2, 3 dioxygenase (IDO). Thus enhanced IDO pathways possibly lead to a reduction in serotonin availability and increase in glutamate receptor activation as a result of metabolism of kynurenine into quinolinic acid. This acid has been observed to enhance glutamatergic receptors [41]. Thus a deficiency in serotonin availability coupled with glutamatergic overdrive in proinflammatory states gives rise towards a likely depressive syndrome [40].

In addition to this, we observed that a decrease in the mean values of MCV, MCH and MCHC occurred among volunteers with depression. Decrease in red cell indices as observed in this study may be an indication of occult nutritional deficiencies among volunteers with major depressive disease investigated in this study. Our observation possibly supports previous studies that suggested a possible correlation between nutritional deficiencies and risk of some neuropsychiatric disorders [42-47]. Previous study has suggested a relationship between low omega-3 fatty acids consumption and the risk of incidence of major depression [42]. Other study has previously linked vitamin B, folate and magnesium deficiencies to depression [48, 49].

Moreover an evaluation of degrees of association between the various parameters studied showed a positive and significant association between haemoglobin and packed cell volume, haemoglobin and RBC, MCV and RBC, Lymphocytes and Monocytes, PT and APTT, PT and FIB APTT and FIB. On the other hand, a negative and significant association was observed between lymphocytes and monocytes, as well as between neutrophils and monocytes. Positive but insignificant associations were observed between Erythrocyte's sedimentation rate. & Protein-C, D-dimer and ESR, and between protein-C and D-dimer. It must be noted that a positive and significant association suggest a direct relationship between the parameters involved whereas a positive and insignificant relationship may suggest the absence of any dependency on the parameters involved. An inverse relationship between the parameters measured suggests that an increase in a particular parameter may leads to decrease in the other parameter. Thus in this study, an increase in prothrombin time test value was also associated with increase in fibrinogen level; suggesting that increase in PT may triggers inflammatory response thus leading to elevated fibrinogen and erythrocytes sedimentation rate values.

The outcome of this study shows major depressive disorder may manifest some degree of inflammation as well as occult nutritional anaemia as demonstrated by elevated values of markers of inflammation (Erythrocytes sedimentation rate, Fibrinogen) and decrease in red cell indices (Mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and mean cell haemoglobin (MCH). There is an elevation in the plasma level of natural inhibitor of coagulation (Protein C) among individuals with major depressive disorder.

Limitation of this Study: this study was limited to haematological and haemostatic parameters in major depressive disorder. The biochemical evaluation was not investigated.

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Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper

Authors' Contribution

OAE conceived and designed the study, contributed to data analysis tools, data analysis and manuscript writing. OTO, AAA contributed to data analysis tools, data analysis and manuscript writing. AOO contributed to data collection and data analysis tools MO, AS, DE contributed to collection of data, data analysis tools and analysis of data. MFO contributed to data analysis tools and analysis of data. All authors approved the final version of the manuscript

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