NEUTROPHILS TO LYMPHOCYTES RATIO AND SOME CYTOKINES IN PATIENTS WITH SCHIZOPHRENIA IN SOUTHEAST, NIGERIA

Abstract

Schizophrenia is likely to be associated with immunological abnormalities. The aim of the study is to investigate the changes in NLR and some cytokines of patients with schizophrenia in Southeast, Nigeria. A total number 100 subjects were recruited for the study comprising 50 for the patients with Schizophrenia and 50 apparently healthy subjects both aged 25 – 60 years. Blood was collected from each subject and levels of cytokines were measured by Enzyme Linked Immunosorbent Assay WBC ($x10^9$/L), Neutrophils and Lymphocyte were measured using manual counting method. Interleukin - 6, TNF-α, WBC and Neutrophil values were significantly higher in Schizophrenic subjects than control subjects (p=0.00, 0.00, 0.00, and 0.001) respectively. Lymphocyte value was lower than control subjects ($p<0.05$). NLR was higher in patients with Schizophrenia than the control. In conclusion, there were significantly higher values in IL-6, WBC and Neutrophil levels of schizophrenic subjects when compared apparently healthy subjects.

INTRODUCTION

"Schizophrenia is a mental disorder characterized by abnormal social behaviour, strange language, and inability to understand reality" (WHO, 2015). "Common symptoms include hallucinations false beliefs, obscure or confused thoughts, inaudible illusions, reduced social involvement and emotional expression, and lack of motivation, with more than 21 million people worldwide are affected" (NIMH, 2015; WHO, 2015). "People with schizophrenia often have additional mental health problems such as anxiety, depression, and substance use disorders" (Buckley et al., 2009). Symptoms usually begin in early adulthood and gradually appear to last for a long time (American Psychiatric Association Mental Disorders Diagnosis and Statistics Manual, 2005). "Schizophrenia is a chronic debilitating disease of unknown etiology. Viral infections and immunopathological responses are among other factors associated with schizophrenia" (Meyer, 2010). "Elevated levels of activation of inflammatory cytokines and microglia may be associated with the pathophysiology of the disease, but anti-inflammatory dysregulation may also play a major role" (Van et al). , 2008; Meyer, 2010; Upthegrove et al., 2014; Tomasik et al., 2016, Petrikis et al., 2017). The cytokine hypothesis

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holds that exposure to increased cytokines in the womb as a result of maternal immunological activation is a substantial risk factor for developing schizophrenia later in life. Numerous epidemiological studies associating repeated infections to the development of schizophrenia support this theory. Furthermore, cytokines play an important role in early neurodevelopment, and variations from the norm can result in aberrant neuroanatomy and brain chemistry. Developmental neuroinflammation has a significant role in rendering the brain vulnerable to morphological and behavioral abnormalities, according to animal models of schizophrenia. Despite the fact that cytokines play a vital role, they are more likely to interact with other risk factors, such as genetic predisposition. New evidence is that cytokine exposure in utero stimulates the brain, and a second adolescent stressor (called a second hit) activates existing developmental vulnerabilities leading to the development of clinical schizophrenia. It suggests that there is a possibility. “Further knowledge of these pathogenic processes and risk factors can be very helpful in reducing the risk of schizophrenia and delaying its onset” (Howard, 2013). “Some reviews have shown that some antipsychotics have anti-inflammatory effects that reduce existing inflammation, whereas atypical antipsychotics have pro-inflammatory effects that may be associated with significant side effects”. Suggests that you have (Drzyzga et al., 2006).

The study was aimed at evaluating NLR and levels of some cytokines of patients with schizophrenia compared to apparently healthy subjects

MATERIALS AND METHODS

Study Site

This study was carried out in Federal Neuropsychiatric Hospital, Enugu State.

Study Subjects and Design

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Total study sample of 100 subjects were recruited in this study by convenient random sampling technique comprising 50 patients with schizophrenia and 50 apparently healthy subjects manifestation.

The tests subjects were aged between the ages of 25 – 60 years who were diagnosed of schizophrenia attending Federal Neuropsychiatric Hospital Enugu. Fifty healthy normal subjects with no history of schizophrenia were recruited for the study age- matched to the test subjects.

Inclusion Criteria

"Already diagnosed as schizophrenic patient who are antipsychotic drug naïve. The diagnosis of schizophrenia was based on the criteria given in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)" (Vinhang Vahia, 2013).

Exclusion Criteria

Schizophrenic pregnant women, subjects who are smokers, subjects on contraceptives, subjects receiving any drug that has the potential of altering inflammatory markers aside the antipsychotic drugs and subjects receiving treatment for other kinds of psychotic, viral or bacterial diseases. Patients with chronic inflammatory diseases and those who used medication that may alter IL regulation (e.g. anti-inflammatory drugs and steroids) were excluded.

Blood Sample Collection and Processing

Eight milliliters of venous blood was collected from the subjects. A 3 ml of the blood sample was dispensed into Ethylenediaminetetraacetic acid (EDTA) bottles for blood count analysis. The remaining sample was transferred into 10 ml plain sample containers all labelled with the subject's name, age and sex (Chang et al., 2010). "The blood sample in the plain containers was spun for 5 minutes at 3000 rpm after allowing the blood to clot for 30 minutes and the

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serum was separated from the red cells using a dry clean Pasteur pipette into a dry clean plain specimen container. The samples were stored at -20^{oC} until analysis. The analysis consist of Human IL-6 quantitation, Human Tumour Necrotic Factor Alpha quantitation by ELISA technique and Blood Count using manual methods" (Chang *et al*., 2010).

Analytical Methods

Interleukin-6 (IL-6)

The human IL-6 ELISA test kit from U-CyTech Biosciences (Cat No CT205A; Lot No 38-28-19-29)

Procedure

All reagents and samples were brought to room temperature before use. Samples were centrifuged again after thawing before the assay. A 100µL of Standard, Blank, or Sample was added per well. The blank well was added with Reference Standard & Sample diluents. Solutions were added to the bottom of micro ELISA plate well, avoiding inside wall touching and foaming as possible. After gentle mixing, the plates were covered with sealer provided and incubated for 90 minutes at 37°C. The liquid of each well was removed, without washing, immediately 100µL of Biotinylated Detection Ab working solution was added to each well and covered with the Plate sealer. After mixing, the plates were incubated for 1 hour at 37°C. After washing three times and decanting, HRP Conjugate working solution (100µL) was added to each well, covered with the Plate sealer and incubated for 30 minutes at 37°C. The wash process was repeated for five times and 90µL of Substrate Solution was added to each well, covered with a new Plate sealer and incubated for about 15 minutes at 37°C. When apparent gradient appeared in standard wells, the reaction was terminated by adding 50µLof Stop Solution to each well. The optical density (OD value) of each well was determined at once, using a micro-plate reader set to 450 nm.

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Quantitation of Human Tumour Necrosis Factor Alpha (TNF-α) **Using Enzyme Linked Immunosorbent Assay** (Chang *et al*., 2010).

The human TNF-α ELISA test kit from U-CyTech Biosciences (Cat No CT209A; Lot No 23-32-12-29).

Procedure

All reagents and samples were brought to room temperature before use. Samples were centrifuged again after thawing before the assay. A 100µL of Standard, Blank, or Sample was added per well. The blank well was added with Reference Standard & Sample diluents. Solutions were added to the bottom of micro ELISA plate well, avoiding inside wall touching and foaming as possible. After gentle mixing, the plates were covered with sealer provided and incubated for 90 minutes at 37°C. The liquid of each well was removed, without washing, immediately 100µL of Biotinylated Detection Ab working solution was added to each well and covered with the Plate sealer. After mixing, the plates were incubated for 1 hour at 37°C. After washing three times and decanting, HRP Conjugate working solution (100µL) was added to each well, covered with the Plate sealer and incubated for 30 minutes at 37°C. The wash process was repeated for five times and 90µL of Substrate Solution was added to each well, covered with a new Plate sealer and incubated for about 15 minutes at 37°C. When apparent gradient appeared in standard wells, the reaction was terminated by adding 50µLof Stop Solution to each well. The optical density (OD value) of each well was determined at once, using a micro-plate reader set to 450 nm.

White Cell Count

Procedure

A 0.38 ml of Turk's solution (crystal violet or aqueous methylene blue) is mixed with 0.02 ml of well-mixed EDTA anticoagulated venous blood and after 2 minutes small amount of this

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mixture is charged onto a Neubauer counting chamber using a capillary pipette held at an angle of about 45^{oc}. The white cells are allowed to settle for about 2 minutes before counting the cells using x10 objectives.

Differential White Cell Count

Procedure

After making a blood smear on a slide the blood film was covered with an undiluted Leishman stain and allowed to be fixed and stained for 2 minutes. Twice the volume of pH 6.8 buffered water was added to the slide and allowed to stain for 10 minutes. The stain was washed off using tap water and left on a rack to air dry.

The film is studied microscopically using x10 and x40 objective lenses for focusing and reading after a drop of immersion oil is placed on the lowest third of the blood film and covered with a clean cover glass. **Statistical Analysis**

The version 22 of the Statistical Package for Social Sciences (SPSS) was used in statistical analysis. The results were expressed as mean (±SD). Comparisons were made using Student's t-test statistical methods were used to test the significant of differences. The results were deemed significant when P<0.05.

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RESULTS

Table 1: Mean ±SD serum levels of some cytokines and some Blood Cell Counts before and after treatment and normal subjects compared using t-test

Parameters	Schizophrenia	Control	P-Value
WBC (10^9/L)	6.66±1.31	5.40±1.07	0.001*
Abs Neutrophils(10^9/L)	4.78±0.35	3.52±0.79	0.027*
Abs Lymphocytes(10^9/L)	1.68±0.30	1.59±0.31	0.005*
NLR	2.76±0.14	2.14±0.11	0.038*

IL-6 (pg/ml)	51.74±14.77	22.04±15.32	0.000*
TNF-α (pg/ml)	52.97±18.52	26.60±26.76	0.000*

KEY:

IL-6= Interleukin 6, TNF-α= Tumour necrosis Alpha, WBC= White Blood Cell

* Statistically significant at 0.05 level of significance

IL-6 levels of patients with Schizophrenia were significantly higher (51.74±14.77 pg/ml) compared to the control (22.04±15.32 pg/ml) (*P=0.00*). TNF-α serum levels of patients with Schizophrenia were significantly higher (52.97±18.52 pg/ml) compared to the control subjects (26.60±26.76 pg/ml) (*P=0.00*). WBC levels of patients with Schizophrenia were significantly higher (6.66±1.31) compared with control subjects (5.40±1.07) (*P=0.001*). Absolute lymphocyte levels of patients with Schizophrenia were significantly higher (1.68±0.30 X 10^9/L) compared with control subjects (1.59±0.31 X 10^9/L) (*P=0.005*). Absolute neutrophil levels of patients with Schizophrenia were significantly lower (4.78±0.35 X 10^9/L) compared with control subjects (3.52±0.79 X 10^9/L) (*P=0.027*). The NLR of patients with Schizophrenia was higher than the control (2.76±0.14, 2.14±0.11, *P=0.038*) respectively.

Discussion

The study showed increase in the neutrophils to lymphocytes ratio (NLR). This is shows high inflammation in patients with Schizophrenia which may the major pathophysiology of the disease and should be used as a marker in this case. Our results are in line with those from previous reports on the presence of increased inflammatory markers in Schizophrenia. However, we discovered higher concentrations of proinflammatory cytokine IL6 and TNFα in patients with Schizophrenia compared to apparently healthy subjects and our finding revealed that there is an elevated levels of IL6 and TNFα in Schizophrenia provides further evidence of a chronic immune activation and inflammatory syndrome in Schizophrenia

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(Potvin et al., 2008). While increased IL6 seems to be state dependent in schizophrenia, TNFα seems to be a trait in patients with Schizophrenia. Elevated TNFα levels have also been previously reported in schizophrenia and have been shown to be associated with symptoms of schizophrenia (Erbagci et al., 2001; Brietzke et al., 2009). "In this study subjects with schizophrenia had significantly higher levels of IL6 and TNFα than controls. Higher IL6 and TNFα levels in subjects with schizophrenia are likely due to inflammation as a result of exposure to the first episode. Elevation of these cytokines is consistent with a well-described pro-inflammatory condition in schizophrenia" (Potvin et al., 2008; Miller et al., 2011). "Increased IL6, a proinflammatory mediator is produced predominantly by macrophages and monocytes. Increased plasma levels of IL6 associated with schizophrenia are confirmed by several metaanalyses" (Potvin et al., 2008; Watanabe et al., 2010 and Miller et al., 2011). "It has been demonstrated that both first episode and chronic schizophrenia patients showed a significant increased level of IL6 and TNFα in comparison to healthy control subjects" (GarcíaMiss et al., 2010; Beumer et al., 2012; Pedrini et al., 2012; Song et al., 2013), suggesting IL6 may play a role in the pathogenesis of schizophrenia. We also found that patients with schizophrenia present a mean increased WBC and neutrophil count value compared with control subjects. This finding is in agreement with work of Zorrilla et al.. (1996) which is in variance with this study. This does not align with the present study which showed significantly lower mean Leucocyte count when compared with the control subjects. Indeed increased values have been described in other mental pathologies (Kronfol et al., 1986).

Conclusion

In conclusion, our study showed that serum IL6, TNFα, leukocyte, and neutrophil levels were significantly higher in drug-naive schizophrenia patients compared to control patients, and IL6 is a pathological marker. , TNFα may be a trait marker.

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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.

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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