

# NEUTROPHIL TO LYMPHOCYTE RATIO AND SOME CYTOKINES IN PATIENTS WITH SCHIZOPHRENIA AFTER ANTIPSYCHOTIC THERAPY IN SOUTHEAST, NIGERIA

## Abstract

The aim of the current study was to evaluate neutrophil to lymphocyte ratio and the levels of IL-6, TNF- $\alpha$  in patients with schizophrenia after six weeks of antipsychotic treatment with Risperidone and Clozapine. Total study sample of 50 subjects of schizophrenia patients and 50 apparently healthy subjects aged 25-60 years were recruited in this study. The cytokines were measured by Enzyme Linked Immunosorbent Assay. The results showed increase in WBC (P=0.001), absolute neutrophil (P=0.000), NLR (P=0.025), IL-6 (P=0.000) and no significant difference in TNF- $\alpha$  (P=0.059) and no significant difference in absolute lymphocyte of schizophrenia patients before treatment compared to after treatment. In conclusion, there were significantly higher values in IL-6, WBC and Neutrophil levels of schizophrenic drug naïve subjects when compared with schizophrenic treated subjects. Tumour Necrosis Factor - alpha serum levels among schizophrenic drug naïve subjects and schizophrenic drug treated subjects showed similar mean values.

**Keywords:** *Neutrophil to lymphocyte ratio (NLR), schizophrenia, antipsychotic drugs, cytokine, interleukin 6 (IL-6), tumour necrosis factor alpha (TNF- $\alpha$ )*

## INTRODUCTION

Schizophrenia is a mental disorder characterized by abnormal social behaviour, strange speech and failure to understand reality (WHO, 2015). Common symptoms include false beliefs, unclear or confused thinking, hearing voices that others do not, reduced social engagement and emotional expression, and a lack of motivation affecting more than 21 million people worldwide (National Institute of Mental Health, 2015; WHO, 2015). People with schizophrenia often have additional mental health problems such as anxiety, depressive, or substance-use disorders (Buckley *et al.*, 2009).

Schizophrenia is a chronic and debilitating disease with unclear aetiology. Among other factors, viral infections and immunopathological responses have been associated with Schizophrenia (Meyer, 2010). Elevated levels of pro-inflammatory cytokines activation may

be implicated in the pathophysiology of the disorder, although anti-inflammatory dysregulations may also play a primary role (Van *et al.*, 2008, Meyer, 2010; Upthegrove *et al.*, 2014; Tomasik *et al.*, 2016, Petrikis *et al.*, 2017).

According to a meta-analysis conducted by Miller *et al.*, (2011), antipsychotic treatment significantly decreased peripheral IL-6 levels in acute relapse or first- episode schizophrenia patients. In that study, IL-6 appeared to be state markers, as they were elevated in acutely exacerbated and first episode patients but were normalized after antipsychotic treatment. In contrast, TNF- $\alpha$  may be considered as trait marker, as the level was increased during acute exacerbations and it also remained increased after antipsychotic treatment. Another meta-analysis by Tourjman *et al.* (2012) showed that antipsychotic treatment increased the plasma levels of TNF- $\alpha$  and reduced the levels of IL-6.

The aim of the current study was to evaluate neutrophil to lymphocyte ratio and the levels of IL-6, TNF- $\alpha$  in patients with schizophrenia after six weeks of antipsychotic treatment with Risperidone and Clozapine.

## **MATERIALS AND METHODS**

### **Study Site**

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

### **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).

### **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. These subjects were excluded by means of questionnaire since literatures has shown that they may be a source of confounding factors which will in turn affect the result output from the study.

## **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. 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 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  $-20^{\circ}\text{C}$  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.*, 2004).

## **Interleukin-6 (IL-6)**

The human IL-6 ELISA test kit from U-CyTech Biosciences (Cat No CT205A; Lot No 38-28-19-29) is used for the in vitro quantitative determination of IL-6 in human fluids such as cell culture supernatant, plasma or serum.

### **Procedure:**

All reagents and samples were brought to room temperature before use. Samples were centrifuged again after thawing before the assay. A  $100\mu\text{L}$  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  $37^{\circ}\text{C}$ . The liquid of each well was removed, without washing, immediately  $100\mu\text{L}$  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  $37^{\circ}\text{C}$ . After washing three times and decanting, HRP Conjugate working solution ( $100\mu\text{L}$ ) was added to each well, covered with the Plate sealer and incubated for 30 minutes at  $37^{\circ}\text{C}$ . The wash process was repeated for five times and  $90\mu\text{L}$  of Substrate Solution was added to each well, covered with a new Plate sealer and incubated for about 15 minutes at  $37^{\circ}\text{C}$ . When apparent gradient appeared in standard wells, the reaction was terminated by adding  $50\mu\text{L}$  of 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.

**Quantitation of Human Tumour Necrosis Factor Alpha (TNF- $\alpha$ ) Using Enzyme Linked Immunosorbent Assay (Chang *et al.*, 2004).**

The human TNF- $\alpha$  ELISA test kit from U-CyTech Biosciences (Cat No CT209A; Lot No 23-32-12-29) is used for the in vitro quantitative determination of TNF- $\alpha$  in human fluids such as cell culture supernatant, plasma or serum.

**Procedure:**

All reagents and samples were brought to room temperature before use. Samples were centrifuged again after thawing before the assay. A 100 $\mu$ L 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 100 $\mu$ L 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 (100 $\mu$ L) 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 90 $\mu$ L 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 50 $\mu$ L of 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 mixture is charged onto a Neubauer counting chamber using a capillary pipette held at an angle of about 45°. 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.

A drop of immersion oil is placed on the lower third of the blood film and covered with a clean cover glass and the film examined microscopically using x10 and x40 objective lens for focusing and reading.

### Statistical Analysis

The version 22 of the Statistical Package for Social Sciences (SPSS) was used in statistical analysis. The results were expressed as mean ( $\pm$ 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  $P < 0.05$ .

## RESULTS

**Table 1: Mean  $\pm$ SD neutrophil to lymphocyte ratio and levels of some cytokines before and after treatment of patients with schizophrenia**

Parameters	Before treatment	After treatment	P-Value
WBC ( $10^9/L$ )	6.36 $\pm$ 1.27	5.32 $\pm$ 1.05	0.001*
Abs Neutrophils( $10^9/L$ )	4.56 $\pm$ 0.35	3.48 $\pm$ 0.79	0.000*
Abs Lymphocytes( $10^9/L$ )	1.61 $\pm$ 0.30	1.62 $\pm$ 0.31	0.346
NLR	2.83 $\pm$ 0.14	2.15 $\pm$ 0.11	0.025*
IL-6 (pg/ml)	51.78 $\pm$ 13.95	42.65 $\pm$ 15.23	0.000*
TNF- $\alpha$ (pg/ml)	52.94 $\pm$ 17.68	57.60 $\pm$ 17.68	0.059

### KEY:

IL-6= Interleukin 6, TNF- $\alpha$ = Tumour necrosis Alpha, WBC= White Blood Cell, NLR= Neutrophil to lymphocyte ratio, Abs= absolute

\* Statistically significant at 0.05 level of significance; p-value = probability value; A p-value of 0.05 was considered significant.

The results showed increase in WBC ( $6.36 \pm 1.2710^9/L$ ,  $5.32 \pm 1.0510^9/L$ ,  $P=0.001$ ), absolute neutrophil ( $4.56 \pm 0.3510^9/L$ ,  $3.48 \pm 0.7910^9/L$ ,  $P=0.000$ ), NLR ( $2.83 \pm 0.14$ ,  $2.15 \pm 0.11$ ,  $P=0.025$ ), IL-6 ( $51.78 \pm 13.95$  pg/ml,  $42.65 \pm 15.23$  pg/ml,  $P=0.000$ ) and no significant difference in TNF- $\alpha$  ( $52.94 \pm 17.68$  pg/ml,  $57.60 \pm 17.68$  pg/ml,  $P=0.059$ ) and no significant difference in absolute lymphocyte of schizophrenia patients before treatment compared to after treatment

## Discussion

The second significant finding of this work is that the 6 weeks of treatment with antipsychotic drugs resulted in significant decrease of IL-6, WBC and Neutrophil levels of after treatment. However, putting the effects of psychotropic drugs on cytokines into perspective, it is possible that the immunosuppressive effects, mediated by decreased monocyte/macrophages functions, may be the reason for the reduced mean IL-6 values and subsequent Th1- Th2 imbalance.

In this study, after treatment, there was no reduction of TNF- $\alpha$  to normal concentrations. These finding is in agreement with the works of Garver *et al.*, 2003; Muller *et al.*, 2010; Chase *et al.*, 2016) which showed no change in TNF- $\alpha$  concentration. This study was in disagreement with the data given by Monteleone *et al.*, which reported decreased TNF- $\alpha$  following ten weeks of treatment and Kim *et al.* (2009) following six weeks of treatment. Also, Dunjic-Kostic *et al.*, 2013 indicated decreased concentrations of TNF- $\alpha$  during acute and remission phase. The study is also in variance with other studies which showed increased serum concentrations of TNF- $\alpha$  after four to six weeks of treatment (Brietzke and Kapczinski, 2008; Drzyzga *et al.*, 2006). Tumour Necrosis Factor-Alpha is a pro-inflammatory cytokine, which is increased in innate immune responses and also during Th1 and Th17 activation. It may also takes part in the pathogenesis of schizophrenia by activating the HypothalamoPituitary-Adrenocortical (HPA) axis, activating-secretion of serotonin as a neurotransmitter and stimulating the indoleamine 2-3-dioxygenase which leads to elimination of tryptophan and activation of kynurenine metabolites, or releasing of the neurotoxic glutamic acid (Himmerich *et al.*, 2009). However, results regarding levels of TNF- $\alpha$  have been less conclusive, reported to be increased, decreased or unchanged (Naudin *et al.*, 1997).

## Conclusion

In conclusion, our study demonstrated that serum IL-6, TNF- $\alpha$ , WBC and Neutrophil levels were significantly higher for drug naïve schizophrenic patients when compared to controls whereas serum mean values of WBC and Neutrophil values were significantly lower after treatment, while TNF- $\alpha$  remained unchanged; however, Lymphocyte values increased significantly after treatment. This demonstrates that IL-6 and TNF- $\alpha$  might play roles in the pathophysiology of Schizophrenia and there has been a growing evidence supporting the role of IL-6 and TNF- $\alpha$  plays on the aetiology of Schizophrenia. This suggest that higher levels of IL-6 and TNF- $\alpha$  reflects a syndrome specific to Schizophrenia. The study also showed that variation in mean values is not gender dependent. These findings in the present study provided preliminary evidence that alterations of TNF- $\alpha$  and IL-6 levels may play a role in schizophrenia suggesting that IL-6 may be a disease state marker and TNF- $\alpha$  a trait marker.

#### **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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