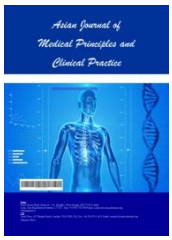

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Neutrophil to Lymphocyte Ratio and Some Cytokines in Pateints with Schizophrenia after Antipsychotic Therapy in Southeast, Nigeria

Emmanuel Ifeanyi Obeagu [a*], Bessie Nonyelum Esimai [b], Loveth Ngozika Ugwu [c], Gina Flor Ramos [d], Stephen Dotun Adetoye [e] and Emmanuel Chukwuma Edupute [c]

[a] Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.
[b] Department of Medical Laboratory Science, Evangel University, Akaeze, Ebonyi State, Nigeria.
[c] Department of Medical Laboratory Science, Madonna University Nigeria, Elele Campus, Rivers State, Nigeria.
[d] Department of Pharmacognosy, Madonna University Nigeria, Elele Campus, Elele, Rivers State, Nigeria.
[e] Ivano Frankivsk National Medical University, Ukraine.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the current study was to evaluate neutrophil to lymphocyte ratio and the levels of IL-6, TNF-α 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 6 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-α (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.

**Corresponding author: E-mail:emmanuelobeagu@yahoo.com;*

Keywords: Neutrophil to lymphocyte ratio (NLR); schizophrenia; antipsychotic drugs; cytokine; interleukin 6 (IL-6); tumour necrosis factor alpha (TNF-α).

1. INTRODUCTION

"Schizophrenia is a mental disorder characterized by abnormal social behaviour, strange language, and inability to understand reality" [1]. "Common symptoms include 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" [2,1]. "People with schizophrenia often have additional mental health problems such as anxiety, depression, and substance use disorders" [3].

Schizophrenia is a chronic debilitating disease of unknown aetiology. Viral infections and immunopathological responses are among other factors associated with schizophrenia [4]. "Elevated levels of inflammatory cytokine activation may be associated with the pathophysiology of the disease, but anti-inflammatory dysregulation may also play a major role" [5-8]. According to a study by Miller et al. [9] Meta-analysis, treatment with antipsychotics, significantly reduced peripheral IL6 levels in patients with acute recurrence or schizophrenia in the first episode. In this study, IL6 was elevated in patients with acute exacerbations and the first episode, but appeared to be a status marker as it normalized after antipsychotic treatment. In contrast, TNFα can be considered a trait marker because it rises during acute exacerbations and remains elevated after antipsychotic treatment. Another meta-analysis by Tourjman et al. [10] Treatment with antipsychotics has been shown to increase plasma levels of TNFα and decrease IL6 levels.

The purpose of the current study was to evaluate the ratio of neutrophils to lymphocytes and the levels of IL6 and TNFα in schizophrenic patients after 6 weeks of antipsychotic treatment with risperidone and clozapine.

2. MATERIALS AND METHODS

2.1 Study Site

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

2.2 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).

2.3 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.

2.4 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°^{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 [11].

2.5 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.

2.5.1 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℃. 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.

2.6 Quantitation of Human Tumour Necrosis Factor Alpha (TNF-α) Using Enzyme Linked Immunosorbent Assay (Chang et al. 2004)

The human TNF-α 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-α in human fluids such as cell culture supernatant, plasma or serum.

2.6.1 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℃. 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.

2.7 White Cell Count

2.7.1 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^{o}c. The white cells are allowed to settle for about 2 minutes before counting the cells using x10 objectives.

2.8 Differential White Cell Count

2.8.1 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.

2.9 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 $P<0.05$.

Table 1. Mean ±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±1.27	5.32±1.05	0.001*
Abs Neutrophils (10^9/L)	4.56±0.35	3.48±0.79	0.000*
Abs Lymphocytes(10^9/L)	1.61±0.30	1.62±0.31	0.346
NLR	2.83±0.14	2.15±0.11	0.025*
IL-6 (pg/ml)	51.78±13.95	42.65±15.23	0.000*
TNF-α (pg/ml)	52.94±17.68	57.60±17.68	0.059

KEY:IL-6= Interleukin 6, TNF-α= 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.*

3. RESULTS

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

4. DISCUSSION

This work revealed 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-α to normal concentrations. These finding is in agreement with the works of Garver et al. [12] Muller et al. [13] Chase et al. [14] which showed no change in TNF-α concentration”. “This study was in disagreement with the data given by Monteleone et al. [15] which reported decreased TNF-α following ten weeks of treatment and Kim et al. [16] following six weeks of treatment”. Also, Dunjic-Kostic et al. [17] indicated “decreased concentrations of TNF-α during acute and remission phase. The study is also in variance with other studies which showed increased serum concentrations of TNF-α after four to six weeks of treatment” [18,19]. “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 Hypothalamo Pituitary-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” [20]. “However, results regarding levels of TNF-α have been less conclusive, reported to be increased, decreased or unchanged” [21,22].

5. CONCLUSION

In conclusion, our study demonstrated that serum IL-6, TNF-α, WBC and NLR 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.

DISCLAIMER

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).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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