International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 9, Issue 9 -2022

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.09.004

Gender-based assessment of tumour necrosis factor – alpha and interleukin – 6 of patients with Schizophrenia in Nigeria

Emmanuel Ifeanyi Obeagu
Department of Medical Laboratory Science, Kampala International University, Western Campus, Ishaka, Uganda.
E-mail: *emmanuelobeagu@yahoo.com*
https://orcid.org/0000-0002-4538-0161

Abstract

Immune mechanisms play a role in the pathophysiology of this schizophrenia and variations in cytokine concentrations have been linked to psychopathology and treatment of schizophrenia which is likely to be associated with immunological abnormalities; however, antipsychotics may induce immune-modulatory effects. The aim of the study is to investigate the changes in serum concentrations of TNF-α and IL-6in schizophrenic patients based on gender. Total study sample of 100 subjects 50 males and 50 females) with *schizophrenia* were recruited in this study by stratified random sampling technique. Blood was collected from each subject and levels of cytokines TNF-α and IL-6were measured by Enzyme Linked Immunosorbent Assay. Comparison of serum level of IL-6 between male and female subjects in drug naïve schizophrenic subjects showed no significant difference (*P=0.51*). Similarly, when mean serum level of TNF-α was compared between male and female subjects, there was no significant difference (*P=0.48*). Comparison of serum level of IL-6 between male and female schizophrenic subjects after 6 weeks treatment showed no significant difference (*P=0.27*). Similarly, when mean serum level of TNF-α was compared between male and female subjects, there was no significant difference (*P=0.18*).

Keywords: Psychotic patients, schizophrenia, interleukin 6, TNF-α

Introduction

Schizophrenia has also been described as a psychiatric disorder characterized by aberrant social behavior, bizarre language, and lack of understanding of reality (WHO, 2015).The main symptoms include false beliefs, unclear or confused thoughts, hearing voices that others cannot hear, decreased social engagement and emotional expression, and lack of motivation. More than 21 million people are affected (NIMH, 2015; WHO, 2015). People with schizophrenia often have other mental health problems, such as anxiety, depression, and substance use disorders (Buckley et al., 2009). Clinical manifestations usually develop slowly, begin in young

adulthood, and persist over a long period of time (APADSMMD, 2005).

Schizophrenia is a chronic, worsening disease of unknown cause. Viral infections and immunopathological responses are associated with schizophrenia, among other factors (Meyer, 2010). Elevated levels of proinflammatory cytokines and microglial activation may be associated with disease pathophysiology, although anti-inflammatory deregulation may also play a major role (Van et al. Meyer, 2010; Upthegrove et al., 2014; Tomasik et al., 2016, Petrikis et al., 2017). There is evidence that antipsychotics reduce proinflammatory markers such as IL-1β, IL-2, IL-6, soluble interleukin-6 receptor (sIL-6R), and TNF-α (Maes et al. al., 1995; Müller et al., 1997; Kowalski et al., 2001), IL-10, soluble interleukin-1 receptor antagonist (sIL-1RA), soluble interleukin-2 receptor (sIL-2R) It increases anti-inflammatory markers such as (Cazzullo et al., 2002). Normalization of inflammatory immune changes may be related to the clinical efficacy of antipsychotic drugs (Meyer, 2010).

Schizophrenia is a chronic, debilitating disease of unknown etiology. Viral infections and immunopathological responses are associated with schizophrenia, among other factors (Meyer, 2010). Elevated levels of proinflammatory cytokines and microglial activation may be associated with disease pathophysiology, but anti-inflammatory dysregulation may also play a major role (Van Berckel et al. Meyer, 2010; Upthegrove et al., 2014; Petrikis et al., 2015; Tomasik et al., 2016). To my knowledge, few studies on cytokine changes after antipsychotic treatment have been published, and the results are inconclusive have been reported to have increased levels of IL-6 and TNF-α (Meyer, 2010; Upthegrove et al., 2014; Petrikis et al., 2017). Levels of IL-6 and TNF-α result in reduced symptoms in drug-naïve patients receiving antipsychotic therapy (Cazzullo et al., 2002; De Witte et al., 2014). The aim of the current study was to assess the levels of IL-6, TNFα, and some blood counts in the same group of subjects before and after six weeks of antipsychotic treatment to assess the effects of these drugs.

Materials and Methods

Study area

The study was done in Africa in Southeast Nigeria at Federal Neuropsychiatric Hospital, Enugu State.

Study Subjects and Design

Total study sample of 100 subjects (50 males and 50 females) with psychotic disorders were recruited in this study by convenient random sampling technique.

Inclusion Criteria

Already diagnosed as an antipsychotic-naive schizophrenic patient. The diagnosis of schizophrenia was based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (VinhangVahia, 2013).

Exclusion Criteria

Pregnant women with schizophrenia, smokers, contraceptives, and people taking medications that may alter inflammatory markers along with antipsychotics, and treatment for other types of psychotic, viral, or bacterial illnesses Patients with chronic inflammatory diseases and those taking drugs that might alter IL regulation were excluded. These subjects were excluded from the questionnaire because the literature has shown that they may be the source of confounding factors that influence the outcome of the study.

Blood Sample Collection and Processing

Eight milliliters of venous blood was drawn from subject. For blood count analysis, 3 ml blood samples were dispensed into ethylenediaminetetraacetic acid (EDTA) bottles. The remaining samples were transferred to plain her 10 mL sample containers labeled with the subject's name, age, and gender, respectively. After allowing the blood to clot for 30 minutes, the blood sample in the plain container was

centrifuged at 3000 rpm for 5 minutes, the serum was separated from the red blood cells using a dry clean Pasteur pipette and placed in a dry clean plain sample container. I put it in Samples were stored at -20 °C until analysis. Analyzes consisted of quantification of human IL-6, quantification of human tumor necrosis factor alpha by ELISA technique (Change 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. After thawing, samples were centrifuged again before testing. 100 µl of standards, blanks, or samples were added per well. Reference standards and sample diluents were added to blank wells. The solution was added to the bottom of the micro ELISA plate wells, avoiding touching the inner wall or forming bubbles. After gentle mixing, the plate was covered with the provided sealer and incubated at 37°C for 90 minutes. Liquid was removed from each well without washing and immediately 100 µl of biotinylated detection antibody working solution was added to each well and covered with a plate sealer. After mixing, the plates were incubated for 1 hour at 37°C. After washing and decanting three times, HRP conjugate working solution (100 µl) was added to each well, covered with a plate sealer and incubated at 37°C for 30 minutes. The washing procedure was repeated five times and 90 µl of substrate solution was added to each well, covered with a new plate seal and incubated at 37 °C for approximately 15 min. When a visible gradient appeared in the standard wells, 50 µl of stop solution was added to each well to stop the reaction. The optical density of each well was immediately measured using a microplate reader set at 450 nm.

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

Procedure

All reagents and samples were brought to room temperature before use. After thawing, samples were centrifuged again before testing. 100 µl of standards, blanks, or samples were added per well. Reference standards and sample diluents were added to blank wells. The solution was added to the bottom of the micro ELISA plate wells, avoiding touching the inner wall or forming bubbles. After gentle mixing, the plate was covered with the provided sealer and incubated at 37°C for 90 minutes. Liquid was removed from each well without washing and immediately 100 µl of biotinylated detection antibody working solution was added to each well and covered with a plate sealer. After mixing, the plates were incubated for 1 hour at 37°C. After washing and decanting three times, HRP conjugate working solution (100 µl) was added to each well, covered with a plate sealer and incubated at 37°C for 30 minutes. The washing procedure was repeated 5 times and 90 µl of substrate solution was added to each well, covered with a new plate seal and incubated at 37°C for approximately 15 minutes. When a visible gradient appeared in the standard wells, 50 µl of stop solution was added to each well to stop the reaction. The optical density of each well was immediately measured using a microplate reader set at 450 nm.

Ethical considerations

After submission of the research proposal, ethical approval was obtained from the hospital's ethics committee after obtaining written consent from the patient's family based on the nature of the patient's health status prior to study initiation.

Statistical Analysis

The version 20 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 method was used to test the significant of differences. The significance level was set at P<0.05.

Results

Table 1: Mean ±SD Serum levels of Interleukin 6 (IL-6), Tumour Necrosis Factor Alpha (TNF-α) and some Blood Cell Counts before treatment between genders compared using t test

Parameters	Male n=20	Female n=10	P value
IL-6 (pg/ml)	50.75±17.21	47.71±8.41	0.51
TNF-α (pg/ml)	49.27±19.27	54.37±17.37	0.48

Comparison of serum level of IL-6 between male and female subjects in drug naïve schizophrenic subjects showed no significant difference (*P=0.51*). Similarly, when mean serum level of TNF-α was compared between male and female subjects, there was no significant difference (*P=0.48*).

Table 2: Mean ±SD Serum levels of Interleukin 6 (IL-6), Tumour Necrosis Factor Alpha (TNF-α) and some Blood Cell Counts between gender after treatment compared using t test

Parameters	Male n=20	Female n=10	P value
IL-6 (pg/ml)	43.87±18.18	36.53±15.33	0.27
TNF-α (pg/ml)	52.14±22.95	63.21±17.12	0.18

Comparison of serum level of IL-6 between male and female schizophrenic subjects after 6 weeks treatment showed no significant difference *(P=0.27*). Similarly, when mean serum level of TNF-α was compared between male and female subjects, there was no significant difference (*P=0.18*).

Discussion

Cytokines are key chemical messengers between immune cells and play important roles in immune regulation. They also play an important role in infectious and inflammatory processes by mediating exchanges between the brain and the immune system, which has recently been the focus of immunological research in schizophrenia (Altamura *et al.,* 2014). The molecular mechanisms underlying increased or decreased serum levels of cytokines in patients with chronic schizophrenia are largely unknown. Previous studies have found abnormalities in pro- and anti-inflammatory cytokines such as TNF-α, IL-2, IL-4, IL-6, IL-10 and IL-18 in patients with schizophrenia. , these changes are shown. May influence psychopathological symptoms of schizophrenia (Potvin*et al.,* 2008; García-Miss *et al.,* 2010; Kunz *et al.,* 2011; Beumer*et al.,* 2012; Pedrini*et al.* ., 2012; Borovcanin*et al.,* 2012; Lv*et al.,* 2014). An imbalance between pro- and anti-inflammatory cytokines may contribute to the pathophysiology of schizophrenia and schizophrenia-like symptoms through various mechanisms. Only the levels of some pro-inflammatory cytokines were measured in the present study. It will also be important to assess levels of anti-inflammatory cytokines in further studies to profile the inflammatory state of Enugu schizophrenia. Changes in serum IL-6 and TNF-α levels in patients with chronic schizophrenia have been previously reported, but they were not different (Kunz et al., 2011; Pedrini *et al.,* 2012),

increased (Naudin *et al.*, 2012; Lin *et al.*, 1998; García-Miss *et al.*, 2010; Beumer *et al.*, 2012) or decreased (Francesconi *et al.*, 2011; Lv*et al.*, 2014; Potvin *et al.*, 2008) were compared with healthy controls. The discrepancy in these results may be due to the small sample size and the relatively mild psychiatric symptoms of acute or chronic schizophrenia. Further follow-up and replication of these results are needed (Ifeanyi, 2020; Obeagu *et al.*, 2022; Obeagu *et al.*, 2022).

Conclusion

The study revealed that there are no changes in the levels of expression of interleukin 6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-α) based on the gender of patients with psychotic disorders.

References

American Psychiatric Association (2005). Diagnostic and Statistical Manual of Mental

Disorders (5th ed.). Arlington: American Psychiatric Publishing. P 555-558.

Altamura AC, Bouli M, Pozzoli S (2014) Role of immunological factors in the pathophysiology and diagnosis of bipolar disorder:comparisom with schizophrenia. *Psychiatry and Clinical Neuroscience*. **68**: 21-36.

Beumer W, Gibney SM, Drexhage RC, Pont-Lezica L, Doorduin J, Klein HC, Steiner J,

Connor TJ, Harkin A, Versnel MA, Drexhage HA (2012). The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes. *Journal of Leukocyte Biology*. **92**: 959-975.

Borovcanin M Jovanovic I Radosavljevic G DjukicDejanovic S Bankovic D Arsenijevic

N Lukic ML (2015). Elevated serum level of type-2 cytokine and low IL-17 in first episode psychosis and schizophrenia in relapse. *Journal of Psychiatric Research*. **46**: 1421–1422.

Buckley PF, Miller BJ, Lehrer DS, Castle D (2009). Psychiatric comorbidities and Schizophrenia. *Schizophrenia Bulletin*. **35**(2): 383-402.

Cazzullo CL, Sacchetti E, Galluzzo A (2002). Cytokine profiles in schizophrenic patients treated with risperidone: a 3-month follow-up study. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. **26**: 33–39.

Chang SH, Chiang SY, Chiu CC *et al.* (2010). Expression of anti-cardiolipin antibodies and inflammatory associated factors in patients with schizophrenia. *Psychiatry Research.* **187**: 341–346.

De Witte L, Tomasik J, Bahn S, Schwarz E (2014) cytokine alterations in first-episodeschizophrenia patients before and after antipsychotic treatment. *Schizophrenia Research*. **154**: 1-3.

Francesconi LP, Cereser K, Mascarenhas RCG (2011) Increased annexin-V and decreased

TNF alpha serum levels in chronic-medicated patients with schizophrenia. *Neuroscience Letters*. **502**(3): 143-146.

Garcia-Miss MR, Perez-Mutul J, Lopez-Canul B, Solis-Rodriguez F, Arankwosky-Sandoval

G (2010) Folate, homocysteine, Interleukin-6, and tumour necrosis factor alpha levels, but not the methylenetetradrofolatereductase C677T polymorphism are risk factors for schizophrenia. *Journal of Psychiatric Research.***44**(7): 441-446.

Ifeanyi, O.E. (2020). Psychiatry – Symptoms, Treatment. Med ClinRev.**7** (2):122

Kowalski J, Blada P, Kucia K, Herman ZS (2001) Neuroleptics normalize increased release of interlukin-1beta and tumour necrosis factor-alpha from monocytes in schizophrenia. *Schizophrenia Research*. **50(3)**: 169-175.

Kunz M, Cereser KM, Goi PD, Fries GR, Teixeira AL, Fernandes BS (2011). Serum levelsof IL-6, IL-10 and TNF-alpha in patients with bipolar disorder and schizophrenia: differences in pro- and anti-inflammatory balance. *Brazilian Journal of Psychiatry*. **33**: 268-274.

Lin A, Kenis G, Bignotti S (1998). The inflammatory response system in treatment-resistant schizophrenia: Increased serum interleukin-6. *Schizophrenia Research.* **32**: 9–15.

Lv L, Gao L, Miller RH (2006). Cytokines and development of the nervous system. In: RM

Ransoroff, EM Benveniste (eds). *Cytokines and the CNS*, 2nd edn. CRC Press, Boca Raton, pp. 93–112.

Maes M, Bosmans E, Calabrese J, Smith R, Meltzer HY (1995) Interleukin-2 and interleukin-

6 in schizophrenia: effects of neuroleptics and mood stabilizers. *Journal of Psychiatric Research*. **29**: 141-152.

Meyer U, Feldon J (2010). Epidemiology-driven neurodevelopmental animal models ofschizophrenia. *Progress in Neurobiology*. **90**(3): 285–326.

Muller N, Riedel M, Ackenheil M, Schwarz MJ (1997). The role of immune function in schizophrenia: An overview. *European Archives of Psychiatry and Clinical Neuroscience.* **249**: 62–68.

National Institute of Mental Health (2015) Schizophrenia. *National Institute of MentalHealth*. **2**: 2650.

Naudin J, Capo C, Giusano B, Mège JL andAzorin JM (1997). A differential role forinterleukin-6 and tumor necrosis factor-alpha in schizophrenia. *Schizophrenia Research*. **26**: 227-233.

Obeagu, E. I., Esimai, B. N., Ugwu, L. N., Ramos, G. F., Adetoye, S. D. andEdupute, E. C. (2022). Neutrophil to Lymphocyte Ratio and Some Cytokines in Pateints with Schizophrenia after Antipsychotic Therapy in Southeast, Nigeria. *Asian Journal of Medical Principles and Clinical Practice*, *5*(4), 47-52.

Obeagu, E. I., Johnson, A. D., Arinze-Anyiam, O. C., Anyiam, A. F., Ramos, G. F. andEsimai, B. N. (2022). Neutrophils to Lymphocytes Ratio and Some Cytokines in Patients with Schizophrenia in Southeast, Nigeria. *International Journal of Research and Reports in Hematology*, *5*(2), 107-112

Pedrini M, Massuda R, Fries GR, de BittencourtPasquali MA, Schnorr CE, MoreiraJC,Teixeira AL, Lobato MI, Walz JC, Belmonte-de-Abreu PS, Kauer-Sant'Anna M, Kapczinski F, Gama CS (2012). Similarities in serum oxidative stress markers and inflammatory cytokines in patients with overt schizophrenia at early and late stages of chronicity. *Journal of Psychiatric Response*. **46**(6): 819–824.

Petrikis P Voulgari PV, Tzallas AT, Boumba VA (2017). Changes in the cytokine profile infirst-episode drug-naïve patients with psychosis after short-term antipsychotic treatment. *Psychiatry Research*. **256**: 378-383.

Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E (2008). Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. *Biological Psychiatry*. **63**: 801–808.

Tomasik J, Rahmoune H, Paul C (2016) Neuroimmune biomarkers in schizophrenia. *Schizophrenia Research*. **176**(1): 3-13.

Upthegrove R, Manzanares-Teson N, Barnes NM (2014) Cytokine function in medication-naïve first episode psychosis: A systematic review and meta-analysis. *Schizophrenia Research*. **155**(1-3): 101-108.

Van Kammen DP, McAllister-Sistilli CG, Kelley ME, Gurklis JA, Yao JK (2008). Elevatedinterleukin-6 in schizophrenia. *Psychiatry Research.* **87**: 129–136.

Van OJ, Kapur S (2009). Schizophrenia. *Lancet*. **374**: 635–645.

Vihang N. Vahia (2013). Diagnostic and statistical manual of mental disorders 5: A quick glance. *Indian Journal of Psychiatry*. **55**(3): 220-223.

World Health Organization (2015) Schizophrenia. Schizophrenia Fact Sheet. **397**: 1

Access this Article in Online

Website: www.ijarbs.com

Subject: Medical Sciences

Quick Response Code

DOI:10.22192/ijarbs.2022.09.09.004

How to cite this article:
Emmanuel Ifeanyi Obeagu . (2022). Gender-based assessment of tumour necrosis factor – alpha and interleukin – 6 of patients with Schizophrenia in Nigeria. Int. J. Adv. Res. Biol. Sci. 9(9): 29-35.
DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.09.004