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TUMOUR NECROSIS FACTOR – ALPHA AND INTERLEUKIN – 6 IN PATIENTS WITH SCHIZOPHRENIA IN ENUGU

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ABSTRACT
Schizophrenia is a chronic and debilitating disease with unclear aetiology, characterized by abnormal social behaviour, strange speech and failure to understand reality, affecting more than 21 million people worldwide. Some studies suggest that immune mechanisms play a role in the pathophysiology of schizophrenia. **Materials and Methods**: In this study, IL-6 and TNF-α levels were evaluated in 30 patients with schizophrenia, before and after treatment and the results were compared with those of apparently healthy volunteers of comparable age and social status. **Results:** The levels of Interleukin – 6 and TNF-α values were significantly higher in Schizophrenic antipsychotic naïve subjects when compared with control subjects. After treatment with antipsychotics, IL-6 showed significantly lower values when compared with schizophrenic drug naive subjects. Tumour Necrosis Factor-α serum levels among schizophrenic treated subjects remained unchanged. It is evident from the study that Schizophrenia can induce the production of IL-6 and TNF-α thus, suggesting that schizophrenia has the tendency to switch the cytokine patterns toward the pro-inflammatory pattern.

KEYWORDS: Schizophrenia, immune mechanisms, IL-6, TNF-α.

INTRODUCTION
Schizophrenia is a chronic and debilitating disease with unclear aetiology[1] characterized by abnormal social behaviour, strange speech and failure to understand reality. It is estimated to affect more than 21 million people worldwide.[2] People with schizophrenia often have additional mental health problems such as anxiety, depressive, or substance-use disorders.[3] Symptoms typically come on gradually, beginning in young adulthood, and last a long time.[4] Elevated levels of pro-inflammatory cytokines and microglial activation may be implicated in the pathophysiology of the disorder, although anti-inflammatory dysregulations may also play a primary role.[5,1,6,7,8] There is increasing evidence for the cytokine hypothesis that exposure to elevated cytokines in utero due to maternal immune activation is a major risk factor for the development of schizophrenia later in life. This is supported by numerous epidemiological studies that connect multiple infections with schizophrenia emergence. Although there is strong evidence for the critical role of cytokines, they most likely work with other contributing risk factors such as genetic predisposition. New evidence indicates that cytokine exposure in utero may prime the brain and that a second stressor during adolescence, referred to as a second hit, may activate existing developmental vulnerabilities resulting in the emergence of clinical schizophrenia. Further knowledge of these pathogenic processes (cytokine expression) and risk factors could be very instrumental in reducing risk and slowing emergence of schizophrenia.[9] Several reviews suggest that some antipsychotics have anti-inflammatory effects reducing the pre-existing inflammation while atypical antipsychotics have pro-inflammatory effects which may be related to significant side-effects.[10,11] Furthermore, there is evidence that antipsychotic medications reduce pro-inflammatory markers such as IL-1β, IL-2, IL-6, soluble interleukin -6 receptor (sIL-6R) and TNF-α[12,13] and increase anti-inflammatory markers such as IL-10, soluble interleukin -1 receptor antagonist (sIL-1RA) and soluble interleukin -2 receptor (sIL-2R).[14] The normalization of pro-inflammatory immune changes may be related to the clinical efficacy of antipsychotic medication.[15]

Few studies have been published on cytokine alterations after antipsychotic treatment and their findings are inconclusive. This study was designed to determine the levels of IL-6 and TNF-α and the values after six weeks of antipsychotic treatment (Risperidone and Clozapine) as a measure of immunological response in adults with schizophrenia. This will add to the existing knowledge of the role of antipsychotics in the management of schizophrenia which is beneficial.

MATERIALS AND METHODS

Study design

This simple random sampling study was conducted at Federal Neuropsychiatric Hospital Enugu (FNHE), Enugu North Local Government Area of Enugu State. Ethical approval was obtained from FNHE Ethical Review Committee. Informed consent was obtained from all subjects' relatives/guardian before the commencement of the study. A total of 50 subjects (aged between 18 and 65 years) were randomly studied. Among these were 30 individuals diagnosed of Schizophrenia and 20 apparently healthy individuals (confirmed history taken).

Blood samples analysis

About 5ml volume of venous blood was collected from the ante-cubital vein of the subjects using standard laboratory collection technique and was transferred into 10 ml plain sample containers all labelled with relevant sample identifiers. 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.

Analytical method

The analysis consist of Human IL-6 quantitation, Human Tumour Necrotic Factor Alpha quantitation by enzyme-linked immunosorbent assay method as described by Ifeanyichukwu *et al*, 2017.[16]

Reagents

The human IL-6 and TNF-α ELISA test kits from UCYTECH BIOSCIENCES Utrecht, Netherlands were used for the in vitro quantitative determination of IL-6 and TNF-α in serum.

Data analysis

Data obtained from the study were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 22 (SPSS Inc., Chicago, Illinois, USA). Continuous data were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA and POST-HOC Tests to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Results

Table 1.0 Mean ±SD serum levels of IL-6 (pg/ml), and serum TNF-α (pg/ml) among Schizophrenic subjects before and after treatment and control subjects.

Parameters	Treatment Naïve Tn (n=30) (mean±SD)	Treatment T1 (n=30) (mean±SD)	Control T0 (n=20)(mean±SD)	F value	P-value
IL (pg/ml)	51.74±15.32	41.42±17.67	22.03± 15.32	20.63	0.000
TNF-α (pg/ml)	53.97±18.52	57.83±21.54	26.60±26.76	13.31	0.000

KEY:
IL-6= Interleukin 6, TNF-α= Tumour Necrosis Alpha, T0 = Control subjects, Tn= Before Treatment, T1= 6 weeks Post Treatment, n= number of participants
There was a significant difference in the mean serum Interleukin (IL-6) and Tumour Necrosis Factor Alpha (TNF-α) levels of Schizophrenic treatment naïve, treatment and control group ($p<0.05$)

Table 2.0: Post Hoc analysis showing serum levels of IL-6 (pg/ml) and TNF-α (pg/ml) among Schizophrenic subjects before and after treatment and control subjects (mean±SD).

Group	IL (pg/ml)	TNF-α (pg/ml)
Control group (T0) n=20	22.03±15.32	26.60±26.76
Treatment Naïve (Tn) n=30	51.74±14.77	52.97±18.52
Treatment (T1) n=30	41.42±17.67	57.83±21.54
F value	20.63	13.31
T0 vs Tn	0.000	0.000
T0 vs T1	0.000	0.000
Tn vs T1	0.04	0.067

KEY: IL-6= Interleukin 6, TNF-α= Tumour necrosis Alpha, T0 = Control subjects,
Tn= Before Treatment, T1= 6 weeks Post Treatment
Tn vs T1 P value = Mean±SD of parameters compared between Schizophrenic drug naïve subjects with Schizophrenic treated subjects using t test.
Tn vs T0 P value = Mean±SD of parameters compared between Schizophrenic drug naive subjects with control subjects using t test.

T1 vs T0 = Mean±SD of parameters compared between treated Schizophrenic subjects and control subjects using t test.

The levels of IL-6 and TNF-α were significantly elevated ($p<0.05$) in patients with schizophrenia when compared with the control subjects. There was significant reduction in levels of Interleukin 6 (IL-6) after treating with antipsychotics while TNF-α remained unchanged after treatment.

Table 3: Mean ±SD Serum levels of Interleukin 6 (IL-6) and Tumour Necrosis Factor Alpha (TNF-α) between male and female drug naïve Schizophrenic subjects compared using t test.

Parameters	Male n=20	Female n=10	P value
IL-6 (pg/ml)	52.75±17.21	49.71±8.41	0.52
TNF-α (pg/ml)	51.27±19.27	56.37±17.37	0.49

Comparison of serum level of IL-6 between male and female subjects in drug naïve schizophrenic subjects showed no significant difference (P=0.52). Similarly, when mean serum level of TNF-α was compared between male and female subjects, there was no significant difference (P=0.49).

DISCUSSION

This study was designed to determine the levels of IL-6 and TNF-α as a measure of cytokine response in adults with schizophrenia. The first part of this study was to determine differences between baseline values of Schizophrenic drug naïve subjects to control subjects. There was a significant higher IL-6 and TNF-α levels in Schizophrenic drug naïve subjects than in control subjects. It is most probable that the higher IL-6 and TNF-α levels in Schizophrenic drug naïve subjects may be due to inflammation as a result of first episode exposure. Elevation of these cytokines is congruent with the well-described pro-inflammatory state in schizophrenia.[17,15] Increased IL-6, a pro-inflammatory mediator is produced predominantly by macrophages and monocytes and increased plasma levels of IL-6 associated with schizophrenia are confirmed by several meta-analyses.[17,18,15] It has been demonstrated that both first episode and chronic schizophrenia patients showed a significant increased level of IL-6 and TNF-α in comparison to healthy control subjects, suggesting that IL-6 may play a role in the pathogenesis of schizophrenia.[19,20,21,22]

The second part of this study was to determine differences between Schizophrenic drug naive subjects and post treatment Schizophrenic subjects. It was observed that after treatment with antipsychotic drugs for six weeks, there was a significant decrease in IL-6 levels. Presently, we cannot clearly explain the mechanism of decreased level of IL-6 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.

Furthermore, there was no reduction in TNF-α level concentrations after antipsychotic treatment when compared to naïve subjects. This finding is in agreement with the works of Muller et al. (2011) and Chase et al. (2016)[15,23] which showed no change in TNF-α concentration after treatment. However, this findings is in disagreement with the data given by Monteleone et al. (1997)[30] following ten weeks of treatment and Kim et al. (2004)[31] following six weeks of treatment, which reported decreased concentrations of TNF-α during treatment phase. This findings is also in variance with other studies which showed increased serum concentrations of TNF-α after four to six weeks of treatment.[24,25] However, results regarding levels of TNF-α have been less conclusive, reported to be increased, decreased or unchanged[32,33,34] and observed discrepancy with some of the previous research results may occur due to different biological material that was analysed (serum vs. plasma vs. CSF), different essay method, small sample size, various phases of disease (acute vs. chronic, active phase vs. remission) and confounding factors such as BMI, smoking, age, and gender.

The mean IL-6 and TNF- α levels should there was no significant difference between males and females when compared with the control group, although gender specific differences were found in previous studies in psychiatric patients such as higher serum levels of TNF-α in females than males[35] or higher IL-6 serum levels in males than in females.[36]

Our results highlight that abnormal immune parameters are present at the onset of psychosis, before pharmacological treatment could control the inflammatory or immune regulation processes. Interestingly, those processes also underlie cognitive disturbances in schizophrenia, which have been related to immune cell populations[26] and inflammatory markers.[27] The molecular mechanisms underlying the elevated or reduced serum levels of cytokines in chronic schizophrenia patients are largely unknown. It is possible that an imbalance between pro-inflammatory and anti-inflammatory cytokines may play a role in the pathophysiology of schizophrenia and the schizophrenia-like symptoms through various mechanisms.[19,28,20,21,24,29]

CONCLUSION

It is evident from the study that Schizophrenia can induce the production of IL-6 and TNF-α, thus, suggesting that schizophrenia has the tendency to switch the cytokine patterns toward the pro-inflammatory

pattern. We also found that antipsychotics significantly normalised the initially abnormal cytokine patterns in schizophrenic patients who were drug naïve, thus suggesting that the normalization of IL-6 was due to the effects of antipsychotic. Also the observed higher levels of IL-6 and TNF-α in acute stage reflects a syndrome specific to Schizophrenia suggesting that IL-6 may be a disease state marker while TNF-α a trait marker. It is important to also evaluate the levels of anti-inflammatory cytokines in further studies, so as to profile the inflammatory state in schizophrenia.

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