ORIGINAL ARTICLE

THE IMMUNOMODULATORY POTENTIAL: ANTIPSYCHOTICS WITH VITAMIN D AND E INTERPLAY ON INTERFERON GAMMA AND TUMOUR NECROTIC FACTOR ALPHA

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Background: Interferon-gamma (IFN-γ) and Tumour Necrotic Factor-alpha (TNF-α) are important immunomodulators raised in psychiatric illnesses. This study is aimed to determine the effects of antipsychotic drugs alone and in combination with vitamin D and E on inflammatory cytokines IFN-γ and TNF-α level in psychotic patients. **Methods:** An ERB-approved (NCT 06200584), randomized control trial was carried out from Jan to Jun 2021 at the Baluchistan Institute of Psychiatry and Behavioural Sciences, Quetta, Pakistan. With non-probability purposive sampling, a total of 260 were enrolled. Group-1 had healthy controls while group-2, 3 and 4 had psychotic patients on olanzapine (10 mg/day), risperidone (2 mg/day) or quetiapine (100 mg/day) respectively. Patients in groups 5, 6 and 7 were kept on recommended risperidone, olanzapine or quetiapine respectively with added vitamin D (200,000 IU once weekly) and Vitamin E (400 mg daily). After two months, the blood samples were analysed for IFN-γ and TNF-α. The results were tabulated with SPSS-26 for 35 patients in each group who completed the study. **Results:** The groups treated with antipsychotics had statistically higher IFN-γ and TNF-α expression than the control group ($p \leq 0.001$). The combination of antipsychotics with added Vitamin D and Vitamin E led to a significant reduction in IFN-γ and TNF-α ($p \leq 0.001$) with maximum decrease with quetiapine in combination with Vitamin D and E. **Conclusion:** Antipsychotics increase IFN-γ and TNF-α expression. The combination therapy across various antipsychotic treatment groups with added Vitamin D and E resulted in lower IFN-γ and TNF-α.

Keywords: Antipsychotics, Vitamin D, Vitamin E, Interferon alpha, Tumour necrotic factor alpha

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INTRODUCTION

Psychiatric disorders have been linked to immune system malfunction. The psychopathology may be exacerbated by the cytokine-regulated inflammatory immunological response. Two important makers, Interferon-gamma (IFN-γ) and Tumour Necrotic Factor-alpha (TNF-α) are raised in psychiatric illnesses, that shows a strong link between inflammation and mental health.[1] IFN-γ, a proinflammatory cytokine, is critical for the immune response.[1–2] Evidence suggests that inflammatory cytokines, such as IFN-γ and TNF-α affect neurotransmitter systems and neurocircuits, leading to severity of symptoms in mental diseases. Patients with significant depression have higher immune markers, including related cytokines supports the relationship between the mental illness and inflammatory mediators.[3,4] Neuroimaging studies show that inflammatory stimuli influence neurocircuits responsible for motivation, motor activity, arousal and anxiety.[5] Monoamines, which include serotonin, norepinephrine, and dopamine, are important neurotransmitters involved in mood regulation. IFN-γ reduces monoamine availability, which explains that why inflammation is connected to treatment resistance in depression. The leading role of TNF-α for the pathophysiology of schizophrenia is in controlling the excitability transmission of neuronal cells and neurotransmitter metabolisms. Its blood concentrations were higher in patients with persistent schizophrenia. Evidence suggests that elevated inflammation is relevant across mental diseases, indicating that therapies targeting inflammation and its downstream effects may have broad application.[6]

Multiple studies have found an intricate link between vitamin D, vitamin E, and inflammatory markers in people with psychiatric illness.[7–8] The associations between 25(OH)D and inflammatory markers were greater in these patients, indicating that vitamin D may have a role in controlling inflammation in the context of depression. Similarly, a study found that vitamin E intake significantly reduced blood concentrations of CRP, IL-6, and TNF-α. Vitamin E, namely α-tocopherol, has been shown in multiple trials having anti-inflammatory properties.[9] These data highlight the effectiveness of vitamin E administration, particularly in certain forms, in reducing subclinical inflammation in adults. The outcomes of several studies revealed further investigations to comprehend the

advantages of combining antipsychotic medications in patients with mental health disorders. These studies offer some insights on administering vitamins in combination with antipsychotic medications to reduce inflammation and no integral research is documented for the use of vitamin D and E to decrease the inflammatory markers in mental illnesses.[10,11] Whether the administration of antioxidant vitamins can reduce important inflammatory markers is yet to be evaluated. This study was designed with the aim to determine the effects of antipsychotic drugs alone and in combination with vitamin D and E on IFN-γ and TNF-α levels in patients with diagnosed psychotic disorders.

METHODOLOGY

This Randomized Control Trial (clinicaltrial.gov, NCT 06200584) was conducted during Jan–Jun 2021 at the Baluchistan Institute of Psychiatry and Behavioural Sciences, Quetta, Pakistan with approval of ERB, Faculty of Pharmacy and Health Sciences, University of Quetta, Pakistan (No. FoP & HS/ 67/22). Based on the prevalence of mental illness in Pakistan 10%, further based on a 95% confidence interval and 5% margin of error using openEpi for sample size calculation resulted in a total of minimum 139 patients.[12] After obtaining written informed consent, 225 diagnosed psychosis patients with DSM-4 TR were enrolled in the study, with 35 healthy controls. These patients aged between 20–70 years, with no other co-morbid, had been taking antipsychotic drugs (quetiapine, olanzapine, or risperidone) with constant doses for at least two months. They were further divided into 6 treatment groups randomly using an online research randomizer (Figure-1). The first group had healthy controls, next three groups (group 2, 3 and 4) on recommended olanzapine (10 mg/day), risperidone (2 mg/day) or quetiapine (100 mg/day) treatment respectively. Rest three groups (group 5, 6 and 7) were kept on olanzapine, risperidone or quetiapine respectively, along with added vitamin D (200,000 IU once daily) and Vitamin E (400 mg BD daily) regime. For two months, all 6 treatment groups got the recommended therapy and then their blood samples were collected.

Expression levels of IFN-γ and TNF-α were determined by polymerase chain reaction. Primers specific to IFN-γ and were designed and synthesized selectively during PCR. The samples for analysis comprised of cell culture, and supernatant serum and plasma were gathered. Serum samples were collected in a serum separator tube (SST) for 30 minutes before centrifugation, whereas plasma samples fetched with anticoagulants were centrifuged within 30 minutes of collection. Cell culture was centrifuged, and aliquot stored at -20 °C to avoid multiple freeze-thaw cycles.

Primer for TNF-α[13]

Forward: **5-CAGGCGGTGCCTATGTCTC-3**
Reverse: **5-CGATCACCCCGAAGTTCAGTAG-3**

Primer for interferon-γ[14]

Forward: **5-CCATCGGCTGACCTAGA-3**
Reverse: **5-GCCACTTGAGTTAAAATAGTTATTCAGAC-3**

The bands found on electrophoresis gel following PCR were quantified using UV transilluminator. All data was tabulated for (n=245) patients who were cooperative and completed the study with equal patients (n=35) in each group, using SPSS-26 for required descriptive and inferential statistics, and $p<0.05$ was considered significant.

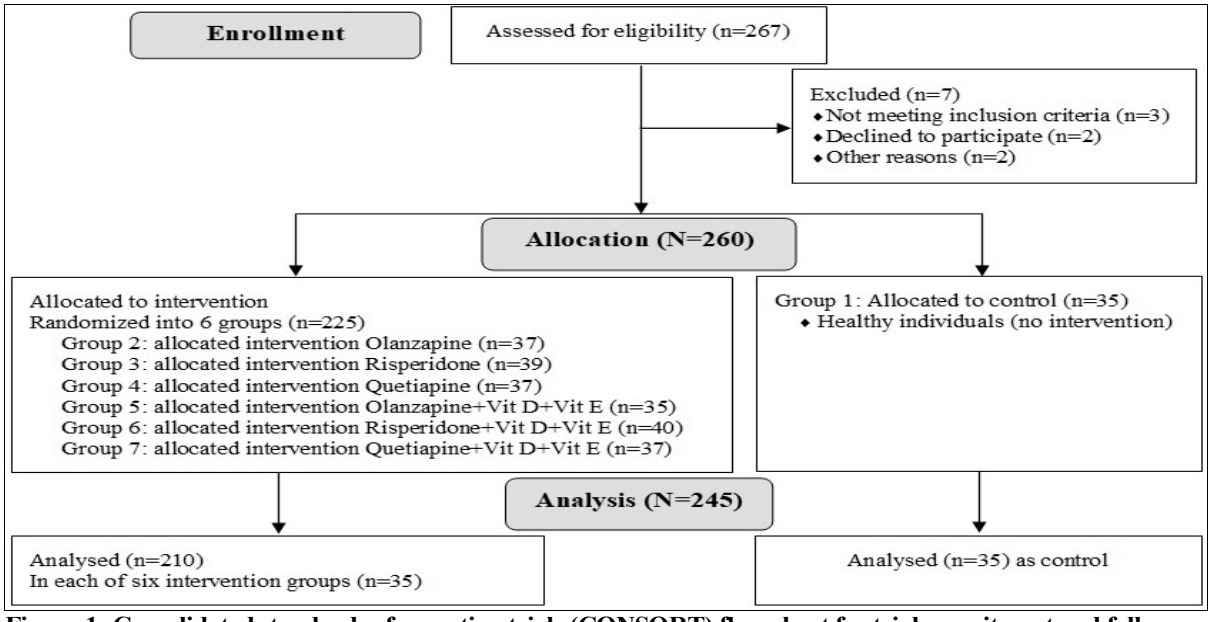

Figure-1: Consolidated standards of reporting trials (CONSORT) flow chart for trial recruitment and follow-up

RESULTS

A total of 245 diagnosed psychotic patients were included in this trial, 150 (61.2%) males and 95 (38.8%) females with mean age 41.64±8.25 years (Range: 25–70 years). TNF-α expression in group 1 was 415.8±12.46, whereas in group 2, 3, and 4 it was 767.22±7.32, 743.01±31.08 and 801.24±2.92 respectively. In groups 5, 6, and 7 it was 383.04±12.62, 227.76±10.64 and 127.8±41.34 respectively. The value for IFN-γ was 424.7±16.88 in control group, and it was 919.4±32.86, 916.6±111.75 and 777.00±28.76 in group 2, 3, and 4 respectively. The values for IFN-γ in group 5, 6, and 7 were 242.3±11.52, 373.1±18.28 and 133.9±10.25 respectively. (Table-1).

The inflammatory marker TNF-α expressions were substantially greater in treatment groups with antipsychotics alone, compared to control (group 1 *vs* 2, group 1 *vs* 3, and group 1 *vs* 4; $p<0.001$). TNF-α expressions were significantly lower in groups 5 ($p<0.005$), and Groups 6, and 7 ($p<0.001$), i.e., antipsychotics with added vitamins D and E compared to control group. When comparison was made between group 2 *vs* 5, group 3 *vs* 6, and group 4 *vs* 7, there were considerably reduced TNF-α levels ($p<0.001$) indicating beneficial effects with added vitamin D and E with antipsychotics. Using ANOVA, group analysis for TNF-α yielded statistically significant outcomes (group 1 *vs* group 2 *vs* group 5, group 1 *vs* group 3 *vs* group 6, group 1 *vs* group 4 *vs* group 7; $p<0.001$). (Table-2).

When the results were tabulated for IFN-γ between the groups (group 1 *vs* 2, group 1 *vs* 3 and group 1 *vs* 4) significantly high ($p<0.001$) expression was observed in treatment groups compared to control group. However, when the respective drugs were given with vitamin D and E (group 5, 6, and 7) the results showed significant reduction in IFN-γ expression ($p<0.001$) than the control group. When results were compared between the groups for IFN-γ expression, it was observed that antipsychotics in combination with vitamin D and E have shown significant lowering the levels of marker than the anti-psychotics alone (group 2 *vs* 5, group 3 *vs* 6, and group 4 *vs* 7; $p<0.001$). The combination of Quetiapine with vitamin E and vitamin D led to a maximum reduction in TNF-α and IFN-γ expression in all the groups. (Table-2).

Table-1: Expression of IFN-γ and TNF-α levels in groups (n=245)

Groups	TNF-α	IFN-γ
G-1: Control	415.8±12.46	424.7±16.88
G-2: Olanzapine	767.2±27.32	919.4±32.86
G-3: Risperidone	743.01±31.08	916.6±111.75
G-4: Quetiapine	801.21±42.92	777.00±28.76
G-5: Olanzapine+Vitamin E+Vitamin D	383.04±12.62	242.3±11.52
G-6: Risperidone+Vitamin E+Vitamin D	227.76±10.64	373.1±18.28
G-7: Quetiapine+Vitamin E+Vitamin D	127.80±41.34	133.9±10.25

Table-2: Comparison of expression of IFN-γ levels and TNF-α between the groups (*p*-values)

Groups	TNF-α	IFN-γ
Gr 1 *vs* Gr 2 (n=70)	<0.001	<0.001
Gr 2 *vs* Gr 5 (n=70)	<0.001	<0.001
Gr 1 *vs* Gr 5 (n=70)	<0.005	<0.001
Gr 1 *vs* Gr 2 *vs* Gr 5 (n=105)	<0.001	<0.001
Gr 1 *vs* Gr 3 (n=70)	<0.001	<0.001
Gr 3 *vs* Gr 6 (n=70)	<0.001	<0.001
Gr 1 *vs* Gr 6 (n=70)	<0.001	<0.001
Gr 1 *vs* Gr 3 *vs* Gr 6 (n=105)	<0.001	<0.001
Gr 1 *vs* Gr 4 (n=70)	<0.001	<0.001
Gr 4 *vs* Gr 7 (n=70)	<0.001	<0.001
Gr 1 *vs* Gr 7 (n=70)	<0.001	<0.001
Gr 1 *vs* Gr 4 *vs* Gr 7 (n=105)	<0.001	<0.001

Independent *t*-test and one-way ANOVA were applied

DISCUSSION

Olanzapine, Risperidone, and Quetiapine groups showed substantially greater IFN-γ and TNF-α mRNA expression levels than the control group. Combination treatments, such as antipsychotics with Vitamin E + Vitamin D significantly reduced IFN-γ and TNF-α expression compared to their respective monotherapies and control group. This suggests that Vitamin E and Vitamin D modulate IFN-γ and TNF-α expression.

Many studies have documented different effects of antipsychotics on inflammatory markers. Kim *et al*[15] stated that all these three above stated antipsychotics reduces IFN-γ levels which is contradictory to our results. Miller *et al*[16] observed that these markers are decreased with antipsychotics, not supporting our analysis. Bapista *et al*[17] documented that Olanzapine decreases TNF-α. Lu *et al*[18], and Kim *et al*[19], also concluded that Quetiapine and Risperidone also decreases TNF-α which is contradictory to our results. Cappuzi *et al*[20] concluded that these inflammatory markers are increases and remained elevated with the use of all antipsychotics alone.

Used with combination with vitamins these drugs reduce inflammatory markers. Valikerin *et al*[21] in his study on Alzheimer patients with psychosis demonstrated that vitamins reduce IFN-γ and TNF-α level in the blood when given with antipsychotics. In similar research by, stated that the inflammatory mediators are raised Panturangi *et al*[22] with antipsychotics and antioxidants. He also narrated that vitamin D insufficiency is common and is linked to poor outcomes for both mental and physical health in these patients. He further added that the effects of vitamin D supplementation on the clinical outcomes of individuals with first-episode psychosis (FEP) is very beneficial. Saboori *et al*[23] concluded in a systemic review and meta-analysis that vitamin E significantly decreases anti-inflammatory markers and cytokines in the blood only when given in dose of 500 mg/day and in patients with elevated levels. The majority of small-scale investigations have raised concerns about the underlying

mechanism and potential overestimation of treatment benefits, although no superiority was seen in predominantly anti-inflammatory medications such as vitamin E over prospective anti-inflammatory agents.[23] All these studies are in favour of our results.

A comprehensive meta-analysis was conducted using data from nine randomized controlled trials to investigate the effects of vitamin D supplementation on mental health and biomarkers of inflammation and oxidative stress in patients with psychiatric disorders. The findings indicated significant positive outcomes, including a reduction in Beck Depression Inventory (BDI) scores, thus showing an improvement in symptoms of depression.[24] Another research demonstrated a substantial reduction in C-reactive protein (CRP) concentrations highlighting an anti-inflammatory effect of vitamin D supplementation. While other biomarkers did not show significant differences, the overall findings suggest that vitamin D supplements may have beneficial effects on mental health and specific markers of inflammation in individuals with psychiatric disorders.[25]

The complex roles of vitamin E, encompassing its antioxidant, anti-inflammatory, and neuroprotective properties, has been identified with a possible connection to major depressive disorder (MDD). Clinical research indicates a link between low vitamin E level and symptoms of MDD and other psychiatric disorders, highlighting the role of vitamin E in regulating oxidative and inflammatory processes linked to depressive symptoms.[26]

There is also strong evidence in literature for vitamin D as antipsychotic and antidepressant agent, with processes involving the control of oxidative stress and neuroinflammation along with a potential dietary supplement for bone health.[27] In a recent meta-analysis by Moslemi *et al*[28], it is expressed that vitamin D significantly reduces inflammatory markers including TNF-α which is in support of our results. Martinaeu *et al*[29] further provided the details that activated form of vitamin D reduces the production and release of IFN-γ which can be beneficial in situations where inflammation is increased.

While these indicators give useful information, they do not provide a complete picture of the underlying processes of inflammation in details. Future research that incorporates other indicators and investigates the molecular mechanisms linked with more inflammatory markers will help to provide a better understanding of the reported effects.

CONCLUSION

IFN-γ and TNF-α levels were significantly elevated in patients treated with Olanzapine, Risperidone, and Quetiapine drugs highlighting the possible effect of antipsychotic drugs on inflammatory markers. Combining vitamin E and vitamin D therapy reduced IFN-γ and TNF-α level indicating that these vitamins may play a role in modulating inflammation linked with mental disorders. Quetiapine along with vitamin D and E proved to be the best combination to achieve minimal inflammatory marker levels in blood.

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