SYNOPSIS

SYNERGISTIC EFFECT OF VITAMIN-E AND VITAMIN-D IN REDUCING RISK OF SIDE EFFECTS ASSOCIATED WITH ATYPICAL ANTIPSYCHOTICS AND IMPROVEMENT OF PSYCHIATRIC ILLNESS

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12th December 2019

List of abbreviations

HDL	High density lipoprotein
LDL	Low density lipoprotein
VLDL	Very low density lipoprotein
SOD	Superoxide dismutase
GPx	Glutathione peroxidase
INF γ	Interferon-γ
S. L P	Serum lipid profile
PCR	Polymerase chain reaction
TNF-α	Tumor necrosis factor alpha

ABSTRACT

Introduction

Depressive disorders and schizophrenia are the serious mental illnesses. Many etiological factors like oxidative stress, socio-economic status, genetic involvement and environmental changes are responsible. Wide range of treatment including anti-depressants and anti-psychotics are available. These drugs especially atypical anti-psychotics causes serious side effects including obesity, changes in serum lipids and blood pressure. In our study we will emphasize the synergistic effects of Vit D and Vit E along with atypical anti psychotics not only in reducing the side effects but also to see the improvement in the disease.

We will be dividing the patients into seven groups. First group will serve as control and other six as treatment groups. Group 2, 3 and 4 will be treated with atypical anti psychotics (olanzapine 10mg/day, risperidone2mg/day, quetiapine 100mg/day respectively) for two months and then same patients will be given Vit D 500- 1000 I.U and Vit E 1000 mg/day along with atypical anti psychotics.

Serum glutathione peroxidase (GPx) and super oxide dismutase (SOD) will be examined by colorimetric method. Tumor necrosis factor alpha (TNF-α) and interferon-γ levels will be analyzed by PCR. Serum lipid profile will be assessed by commercially available kits. Improvement in illness will be assessed through Positive and Negative Syndrome Scale score (PANSS)

OBJECTIVES:

1. The objective of this study is to examine the safety and efficacy of vitamin D and vitamin E in the treatment of psychiatric disorders.
2. To determine the improvement and reversibility of side effects of psychiatric disorders.

MATERIAL AND METHODS:

Study design and setting: It will be an interventional prospective case control study and it will be conducted in the Department of Pharmacology, in collaboration with Psychiatry Department JPMC, Karachi.

Study period: Two years after approval of synopsis.

Study sample size: Based on prevalence with confidence interval 99% and bond of error 1% using online software www.openepi.com, my sample size is **240**

Sampling technique: Non probability purposive sampling will be used.

Ethical Approval: Ethical approval will be taken from IRB, JPMC, Karachi

Result: Results will be evaluated and compared statistically between and within groups.

Keywords: Anti- Psychotics, Olanzapine, Quetiapine, risperidone, tumor necrosing factor alpha, interferon gamma, positive and negative syndrome scale

INTRODUCTION:

Mental illness is defined as any disturbance of emotional equilibrium, as manifested in maladaptive behavior and impaired functioning, caused by genetic, physical, chemical, biologic, psychologic, or social and cultural factors (Singh et al., 2017). Mental illness is a major problem all over the world. Studies from developed countries have reported a prevalence rate of 15 per 1000 population (Jayanthi et al., 2013). In Pakistan the reported rate is 73 per 1000 population (Sarumathy et al., 2014). Organic psychosis (0.4%), schizophrenia (2.7%) and affective disorders (12.3%) contribute to a rate of 15.4% for psychosis. The prevalence rate for mental retardation (6.9%), epilepsy (4.4%), neurotic disorders (20.7%), alcohol/drug addiction (6.9%) and miscellaneous group (3.9%) were estimated (Pakpoor et al., 2014). Different treatment modalities such as drugs, electro-convulsive therapy, counselling, psycho-surgery and psychotherapy are used in the treatment of mental illness. (Sarumathy et al., 2014)

An adverse drug reaction (ADR) is defined by the World Health Organization (WHO) as "any response to a drug which is noxious and unintended, and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function" (Singh et al., 2017).

Patients with psychiatric illness needs lifelong therapy with psychotropic drugs which predisposes them to an array of ADRs.(Sarumathy et al., 2014). The common adverse effects associated with psychotropic drugs are weight gain, somnolence, tremors, and tardive dyskinesia. These adverse effects tend to deteriorate the mental and physical well-being of the patient and thus lead to patient non-adherence to therapy (Sarumathy et al., 2014)

Antipsychotics are a class of drugs used in the treatment of psychiatric disorders. Since the introduction of antipsychotic medication for the treatment of psychosis, a wide range of different types of drugs have been developed under this genre. The first generation of antipsychotic medication is known as the 'typical antipsychotics' and these were first discovered in the 1950s. Soon following their clinical use it was recognized that they caused extrapyramidal symptoms (EPS) in patients including parkinsonism, tardive dyskinesia, akathesia and dystonia. (Pakpoor et al., 2014).

Antipsychotics

Antipsychotics are associated with a range of side effects. It is well recognized that many people stop taking these drugs (around two thirds even in controlled drug trials) due in part to adverse effects.(Tiwari et al., 2012). The typical antipsychotics has adverse effects like extrapyramidal reactions including acute dystonias, akethesia, parkinsonism (rigidity and tremor), tardive dyskinesia, tachycardia, hypotension, impotence, lethargy, seizures, intense dreams or nightmares, and hyper-prolactinemia (Tiwari et al., 2012). On the other hand, the atypical antipsychotics causes obesity, alter lipid profile and subsequent metabolic syndrome specially by Olanzapine, Risperidone and Quetiapine (ketzang 2004). Side effects from antipsychotics can be managed by a number of different drugs. Continuous use of neuroleptics has been shown to decrease total brain volume by 10% in macaque monkeys.(Dorph-Petersen et al., 2005

Role of Vitamin D

Vitamin D deficiency is prevalent throughout the world, particularly in high-risk groups including pregnant woman, infants, dark-skinned migrants and the elderly (Lips et al., 2010). In Australia, a recent study showed that 31% of the population has vitamin D deficiency, which is defined as a serum concentration of 25-hydroxyvitamin D [25(OH)D] below 50 nmol/L (Daly et al., 2012). Although vitamin D is essential for calcium homeostasis and bone metabolism, it also has a role in other physiological functions, such as an immune modulator (Baeke et al., 2010) and in cell proliferation and differentiation (Bikle et al., 2009). Research over the past 15 years has revealed many functions of vitamin D in brain development and adult brain function (Harms et al., 2011). More recently, evidence has accumulated to suggest that low vitamin D levels during adulthood may also be associated with adverse brain-related outcome. A growing body of evidence from epidemiology and neuroscience links vitamin D deficiency with a range of neuropsychiatric disorders and neurodegenerative diseases (Eyles et al., 2013). Low vitamin D would have utmost public health implications and treatment is safe, cheap and publicly acceptable.

Adequate vitamin D status is also needed for optimal muscular strength in youth (HELENA study) and the elderly.(Hirani et al., 2013). In addition to these diseases, vitamin D has also been linked to mental illnesses including Alzheimer's (Balion et

al., 2012) premenstrual mood disorder, (Eskandari et al., 2007) major depression and psychosis. (Gracious et al., 2012). However, the relationship of vitamin D status to the functions of the brain is also in its infancy (Gracious et al., 2012) with some evidence of negative, cognitive(Annweiler et al., 2010) and mood(Graham et al., 2015) effects in people with insufficient vitamin D levels. Neuro-protective qualities of vitamin D have been observed in many studies, and recent reviews have suggested that vitamin D could play an important role in the central nervous system for healthy neural development and function. (Wrzosek et al.,2013; Deluca et al., 2013). A correlation between schizophrenia and low prenatal and early life vitamin D status has been well demonstrated (McGrath et al., 2010) and supplementation could be important for disease prevention. (Wrzosek et al., 2013)

Although several studies have shown metformin to be effective, this still hasn't been included in clinical guidelines on managing antipsychotic side effects. Several RCT which evaluated the efficacy of metformin in treating antipsychotic induced weight gain have been published recently (de Silva et al., 2015; Jarskog et al., 2013). Therefore it is important that the evidence regarding metformin is synthesized.

Study conducted in New Zealand, in which they observed severe vitamin D deficiency in schizophrenia and other psychiatric illnesses.(Menkes et al., 2012). Vitamin D deficiency and associated psychosis has been noted in dark-skinned immigrant populations. In a retrospective chart review of 18 first generation immigrants from Africa and Haiti with acute psychosis, all patients had vitamin D levels in the insufficient range. (Dealberto et al., 2013), however many of the clinical descriptions of the episodes were not consistent with a schizophrenia diagnosis, and only seven of these patients went on to be diagnosed with schizophrenia. Similarly, in a cross-sectional study of Norwegian patients, vitamin D deficiency was present in 80% of the psychotic immigrant population with dark complexions. Among native-born Norwegians with psychosis, 43% had vitamin D deficiency and had lower serum vitamin D levels compared to reference sample (Berg et al., 2010). In a cross-sectional study of 35 adolescent inpatients, 33.7% patients were vitamin D deficient; of those deficient 40% exhibited psychotic features compared to 16% of patients who were not vitamin D deficient. (Gracious et al., 2012). In a case-control study, Crews

found significantly lower levels of serum vitamin D among 69 first-episode psychosis inpatients compared to matched healthy controls (Crews et al., 2013). In another small study of first-episode patients, more severe negative symptoms and cognitive impairment were correlated with lower vitamin D levels. These patients had not received more than a total of four months of antipsychotic treatment (Graham et al., 2015) Finally, in a recent meta-analysis, Belvederi Murri et al. found that patients with psychotic disorders (mainly schizophrenia) had consistently lower vitamin D levels compared to healthy controls (Murri et al., 2013) The authors examined 7 studies overall for a total of 523 patients and 7545 controls. Of note there was heterogeneity of effect size, and most studies were case-control or cross-sectional. As with all observational studies, we emphasize that the results have to be taken with caution, given there may be many potential confounding variables that are present in patients with schizophrenia and also affect vitamin D levels such as insufficient nutrient intake or little sunlight exposure.

Vitamin D deficiency early in life may also contribute to schizophrenia risk. McGrath et al. examined a Danish cohort of over 400 patients with schizophrenia and carefully matched controls in which neonatal dried blood spots had been collected (McGrath et al., 2010). Among individuals in the lowest quintiles of neonatal vitamin D there was a two-fold elevated risk of schizophrenia compared to those in higher quintiles. Interestingly, those neonates with the highest vitamin D levels also had an increased risk of schizophrenia. In a birth cohort of over 2000 people in the UK, Sullivan et al. did not find an association between maternal vitamin D levels and risk of psychotic illness at age 18 (Sullivan et al., 2013). However, it is likely that some individuals at this age have not yet experienced onset of the illness; it will be important to follow the cohort over the subsequent years.

Intervention studies have had mixed results. In a Finnish birth cohort of over 9000 people, vitamin D supplementation with at least 2000 IU/day in males in the first year of life reduced the risk of schizophrenia by 77% (RR .23) compared to those receiving less than 2000 IU/day; these findings did not hold true in females(McGrath et al., 2004). Dealberto et al., 2013 hypothesized that early vitamin D supplementation is crucial for pro-differentiating signals in the developing brain, and potentially also

for normal brain recovery after insult. In the study of immigrant population discussed above, provided daily vitamin D supplementation of 1000 IU/day in addition to ongoing antipsychotic treatment, and concluded there were no changes in psychiatric symptoms after vitamin supplementation.

Role of Vitamin E

Vitamins E is an antioxidants that protects against cellular damage due to inflammation or highly reactive oxygen-containing molecules. In another small study among 14 in patients with schizophrenia in India, plasma vitamin E and C levels were significantly lower when compared to control subjects (D'Souza et al., 2003)

One study reported a decrease in the Brief Psychiatric Rating Scale (BPRS) and Positive and Negative Syndrome Scale (PANSS) scores among patients on antipsychotic treatment after supplementation with vitamin E (Avindakshan et al., 2003); however given the combination treatment it is difficult to conclude what effects are due to vitamin supplementation alone. A randomized placebo-control study by Bentsen et al 2013 also supplemented vitamin E (1000 mg/day) to patients with schizophrenia on antipsychotic medication; among those patients with low red blood cell polyunsaturated fatty acids (PUFAs), the vitamin supplementation actually impaired recovery from acute psychosis compared to placebo. The authors hypothesize that vitamin E at a high enough dose can act as a pro-oxidant if there is inadequate antioxidant activity, thus increasing oxidative stress; it could also inhibit the beneficial γ- and δ tocopherols. Ten of the thirteen patients showed improvement on CGI scores and plasma vitamin C levels increased over the eight weeks of treatment. The authors report that most of the symptomatic improvement occurred in the positive symptom domain. Notably, there was no control group in this study, and the weekly study visits with medical staff may also contribute to improvement in psychotic symptoms.

Need for the Study

Patients suffering from physical illness are given specific treatment because the causes are specific and the signs and symptoms are also specific. In a psychiatric setting the treatment may not be so specific and most patients are given more than one treatment. These treatment methods vary from patient to patient. Some patients do not want treatment and may not co-operate with the doctors because of hazards of side effects. Some do not realize that they are ill and may actively resist all forms of treatment. To minimize side effects, doses should be increased slowly until there is a therapeutic effect, side effects emerge, or the maximum recommended dose is reached. It may take 6 to 8 weeks for effective results, or as determined by the practitioner. Each drug has its own set of actions and side effects, some more serious than others; these should be evaluated in terms of each user's medical status, including interaction with other medications. Consider the effect of one psychotropic medication at a time.

A study was conducted on "psychotropic side effects of commonly prescribed medications in the elderly" are prevalent and, in most instances, predictable and preventable. They are also associated with considerable morbidity and mortality. Delirium, mood changes, and psychotic symptoms are the most serious categories of psychotropic side effects. Although any drug that crosses the blood-brain barrier has the potential for causing psychotropic side effects, certain commonly prescribed classes of drugs, such as anticholinergics, psychotropics, antihistaminics, and many over-the-counter medications, are particularly suspect. A variety of interventions, such as decreasing the inappropriate prescription and performing a routine evaluation of the older patient's drug regimen, can prevent or minimize psychotropic side effects. Practitioners must be vigilant in monitoring patients for psychotropic side effects of all commonly prescribed drugs.

A study was conducted on "Educating and informing patients receiving psychopharmacological medications. Are family physicians in Pakistan up to the Task? A total of 354 adult patients were interviewed during 3 days. Among them, 73 (20.6%) were prescribed psychopharmacological medications. Among patients receiving psychopharmacological medicines, 37 (50.7%) did not know their diagnosis; 50 (68.5%) were unaware of the disease process; 52 (71.2%) were unaware

of alternative treatments; 63 (86.3%) were not cautioned about the potential adverse effects of the drugs; 24 (32.9%) were unaware of the duration of treatment and in 60 (82.2%) of the participants an appropriate referral had not been discussed. For all aspects of education, patients prescribed psychopharmacological medications knew less as compared to those patients that were prescribed other medications.

When a person knows the side effects of the medication he/she is taking, he/she is better equipped to make a decision about whether this is an appropriate medical treatment for her. The side effects of some treatments may make some people decide not to take the medication or to pursue another treatment option. If a medical emergency or serious complication arises as a result of medicinal treatment, a person who is aware that this is a possible side effect of the medication can more quickly seek appropriate medical treatment. People who are taking medications that have chronic but not necessarily serious side effects can learn strategies to manage the side effects in our population. Vitamin D and Vitamin E are pro-hormone with skeletal and extra skeletal properties that could potentially reduce the severity of these side effects. Their role as an adjunctive therapy for the management of side effects of drug agents has not been adequately studied. Effective strategies to curb these side effects will improve the overall health of patients with psychiatric illnesses who receive antipsychotics drugs. This is the reason the researcher selected this topic for the study.

OBJECTIVES:

The objectives of this study are:

1. The objective of this study is to examine the safety and efficacy of vitamin D and vitamin E in the treatment of psychiatric disorders.
2. To determine the improvement and reversibility of side effects of psychiatric disorders.

HYPOTHESIS OF THE STUDY:

Null Hypothesis

There is no statistically significant difference of Synergistic effect of Vitamin E and Vitamin D in reducing risk of side effects associated with atypical anti psychotics

Alternate Hypothesis

There is statistically significant difference of Synergistic effect of Vitamin E and Vitamin D in reducing risk of side effects associated with atypical anti psychotics

OPERATIONAL DEFINITIONS:

Vitamin D level: It will be Based on laboratory reference values, Vitamin D severe deficiency, deficiency, insufficiency, and sufficiency were defined as a 25-hydroxy Vitamin D (25(OH)D) levels <10 ng/mL, 10–20 ng/mL, 21–30 ng/mL, and >30 ng/mL, respectively.

Vitamin E level: It will be based on below Reference Values:

- 0-17 years: 3.8-18.4 mg/L
- or =18 years: 5.5-17.0 mg/L
- Significant deficiency: <3.0 mg/L
- Significant excess: >40 mg/L

Atypical Antipsychotic drugs:

In this study it refers to, a class of drugs used to control psychotic symptoms in patients with psychotic disorders such as schizophrenia, severe depression, and delusional disorder that is, atypical [Risperidone, Olanzapine, quetiapine]

MATERIAL AND METHODS:

Study design:

It will be interventional prospective case control study.

Study period:

Two year after approval of synopsis.

Study setting:

Study will be conducted in the Department of Pharmacology with collaboration of Psychiatry department JPMC, Karachi

Study sample size:

Based on the prevalence of psychiatric illnesses10.0%a total of 240 patients will be selected based on 99% confidence interval and 1% margin of error using online software www.openepi.com The sample size estimated is 240 (Goswami et al., 2014)

Sampling technique:

Non probability purposive sampling technique will be used.

SAMPLE SELECTION:

Inclusion Criteria:

1. Participants who were under antipsychotic therapy such as olanzapine, risperidone and quetiapine.
2. Participants who were between the ages 20 – 70 years both sex male and female.
3. Participants who were taking a combination of one or two antipsychotics.
4. Participants who were under antipsychotic therapy
5. Patients having vitamin-D and vitamin-E levels below normal

Exclusion Criteria:

1. Psychiatric patients those who were pregnant and nursing mothers.
2. Participants who have renal and hepatic failure or parathyroid disorders.
3. Patients who use phosphorous, calcium or vitamin D supplements, or had co-administered medications of anticonvulsants, anti-fungal and corticosteroids with history of other psychiatric and neurologic disorders.
4. Any metabolic syndrome

Data collection procedure:

During the study period, 240 were will be registered into the study who selected according to the inclusion and exclusion criteria for psychiatric disorders with baseline serum vitamin D and vitamin E concentrations. All patients will be on antipsychotics for at least two months and a stable dose for at least 2 month will be

included in the study and the antipsychotics dose remained unchanged during the study period.

Patients groups:

All the patients will be equally divided (n = 35) into seven groups as:

Group 1: Treatment group with Resperidone

The patients who will receive 2 mg/day Risperidone (*n* =35) at baseline along and will be followed for 2 months will be known as the intervention group.

Group 2: Treatment group with Olanzapine

The patients who will receive 10 mg/day Olanzapine (*n* =35) at baseline along and will be followed for 2 months will be known as the intervention group.

Group 3: Treatment group with Quetiapine

The patients who will receive 50-100 mg/day Quetiapine (*n* =35) at baseline along and will be followed for 2 months will be known as the intervention group.

Group 4: Treatment group with Vitamin D + Vitamin E + Risperidone

The patients who will receive 5000 IU per week plus vitamin E 1000 mg per day plus 2mg Risperidone (*n* = 35) at baseline along with their antipsychotic regimen and followed for 2 months will be known as the intervention group.

Group 5: Treatment group with Vitamin D + Vitamin E + Olanzapine

The patients who will receive from 5000 IU per week plus vitamin E 1000 mg per day plus 10 mg Olanzapine (*n* = 35) at baseline along with their antipsychotic regimen and followed for 2 months will be known as the intervention group.

Group 6: Treatment group with Vitamin D + Vitamin E + Quetiapine

The patients who will receive from 5000 IU per week plus vitamin E 1000 mg per day plus 100 mg Quetiapine (*n* = 35) at baseline along with their antipsychotic regimen and followed for 2 months will be known as the intervention group.

Group 7: Control group

Twenty apparently age and sex matched controls that will come for routine checkup, will be included from outpatient department.

All the studied groups received mentioned treatment for 2 months. All psychiatric patients will be managed based on the guidelines of the American Psychiatric

Association (18). All patients will beexamined clinically, and their demographic and menstrual and medical history will be recorded by a trained nurse using a check list.

Sample collection and measurement

A sample of 10 mL of venous blood will be collected into EDTA tube that will contain anticoagulant at the baseline by the end of the 2nd month (Graham et al., 2015). Each sample will be centrifuged (3500×g) for 15 min.

ASSESEMENT OF VIT D3 AND VIT E

Then the serum will be separated and the serum Vitamin D and Vit E concentration will be analyzed using a commercially available 25-OH Vitamin D and Vit E ELISA kit.

ASSESMENT OF PSYCHIATRIC IMPROVEMENT

For each patient, a set of variables will be collected including demographics (age, gender, marital and smoking status, duration of disease, and initial Positive and Negative Syndrome Scale score (PANSS) [(20).]The PANSS assessment will be blind and made by two trained and expert psychologists independently. The whole study will be consisted of 2 visits: a baseline, and a follow up visit after 2 months of treatment. At baseline visit, blood will be collected for MDA, vitamin D, and vitamin E levels assessment. All patients will receive standard routine medical care throughout the study. Regularity of drug intake will be ensured over telephone, and with patient's compliance sheet.

Assessment of lipid profile

The metabolic panel includes a lipid profile for the measurement of total cholesterol (TC), triglycerides (TAG), high density lipoproteins (HDL), low density lipoproteins (LDL), and very low-density lipoproteins (VLDL).

Determination of Glutathione Peroxidase and Superoxide Dismutase Activity

As the oxidative stress of tissue generally involves the Glutathione Peroxidase and Superoxide Dismutase system, the levels of serum Glutathione Per-oxidase and Superoxide Dismutase will be measured in each group by colorimetric method using commercially available kits.

Anti-inflammatory Markers

TNF α and interferon gamma expression levels will be done though PCR Appropriate primers for TNF-α and IFN-γ will be synthesized and used for the production of copies by PCR

Primer for TNF-α (Wang et al., 2003)

Forward 5-CAGGCGGTGCCTATGTCTC-3

Reverse 5-CGATCACCCCGAAGTTCAGTAG-3

Primer for interferon-γ (Harris et al., 2005)

Forward 5-CCATCGGCTGACCTAGA-3

Reverse 5-GCCACTTGAGTTAAAATAGTTATTCAGAC-3

INSTRUMENTS

Brief Psychiatric Rating Scale (BPRS).

This instrument has 16 items each scored on a seven points scale. For example, absent, minimal, mild, moderate, moderate-severe, severe, and extreme. The items include anxiety, emotional withdrawal (autism), depressive mood, guilt feeling etc. There are criteria to define the symptomatology items but not the severity ratings. It is suitable for rating of sever psychiatric illness but not minor disorders.

Positive and negative syndrome rating scale (PANSS)

This scale rates blunted effect, poor rapport, social withdraw difficulty in abstract and stereo typed thinking and lack of spontaneity. A thirty minutes interview is required. It has seven items for positive symptoms and seven items for negative symptom and scoring is the same as in BPRS.

Extra pyramidal symptom rating scale (EPSRS)

On which quantitative rating of the symptoms of parkinsonism, dystonia and dyskinesia is done. It has twelve items each scored on four points scale.

Quality of life questionnaire (WHO QOL-BREF)

Quality of life is a general term applied to the totality of physical, psychological and social functioning. It is determined by physical impairment,

emotional reaction, personality, illness etc. QOL scale requires great deal of concentration and responsibility and assessed very carefully as involves many diverse aspects and is laborious. It has 26 items each scored on five-point scale. Increase in total count means an improvement in the symptoms and hence in the quality of life.

Ethical considerations:

The study will be undertaken after an ethical approval for the current study will be taken by the Institutional Review Board (IRB) of Basic Medical Sciences Institute, JPMC, Karachi. Data which will be obtained during the study will be kept extremely confidential.

In a psychiatric study it would always be necessary to ask and discuss about some sensitive issues, regarding personal and family affairs. But extreme precaution would be taken not to break the limits of ethical issues and not to harm physically, psychologically socially and spiritually to the participants, during the course of thesis work.

Points to preserve the ethical issues-

1. This study will not involve one's body organ or fetal tissues.
2. Precaution will be taken to ensure one's anonymity.
3. Subject or key relatives will be clearly informed about the scope and limitation of the study.
4. A written consent will be taken from the subject and / or from the key relatives if subjects are minor or unable to give reliable information (and for that purpose a consent form was supplied).
5. The patient and or relatives who will be unwilling to participate will be excluded from the study.
6. Precautions will be taken to maintain ones confidentiality of personal data and obscurity.
7. No financial involvement of the client or respondents will be encouraged.
8. Basic human rights to refuse, or to accept will be maintained.
9. During the study period no medication will be given to the patient for trial excepting therapeutic measures provide by a psychiatrist.

During the course of this study Precautions will be taken about not to produce any environmental hazards or breach.

Statistical analysis:

Data will be processed and analyzed using computer software SPSS (Statistical Package for Social Sciences) version 22.0. Data will be assessed for test of normality in SPSS.Qualitative variables will be recorded according to frequency and percentage and quantitative variables in terms of Mean ± SD (Standard Deviation). To compare continuous variables in five groups, independent sample t-test will be applied and to compare categorical variables Chi- Square or Fisher›s exact test will be used. Kruskal-Wallis test will be used, for comparing The correlation between serum vitamin D and vitamin E level and negative and positive symptoms at baseline will be made by Pearson correlation test. All the data will be calculated on 99% confidence interval. P value $\leq$ 0.05 will be considered significant level for all the comparisons.

Synergistic effect of Vitamin E and Vitamin D in reducing risk of side effects associated typical anti psychotics and improvement of psychiatric illness

Questionnaire

1. Identification Number: _____

1.1 I.D Code: _____

1.2 Name:_____

1.3 Father's/ Husband's Name:_____

1.4 Sex: 1. Male 2. Female

1.5 Marital Status:_____

1.6 Date of Birth (dd/mm/yy): _____

1.7 Age (yr): _____

1.8 Mailing address: _____

_____

1.9 Permanent address: _____

_____

1.10 Religion: _____

1.11 Nationality: _____

1.12 Duration of illness:_____

2. Personal history:

2.1 Area of residence: 1. Rural 2. Urban 3. Sub-Urban

2.2. Education: 1. Illiterate 2. Primary 3. Middle

4. Matriculation 5. Intermediate 6. Graduate

7. Masters or above

2.3.**Occupation:** 1. Professional/Managerial/ Business 2. Clerical

3. Technical 4. Skilled worker

5. Unemployed/ Pensioner 6. Housewife

7. Labour 8. Govt Job

2.4. Socio economic status 1. Lower 2. Middle 3. Upper

3. Previous history of psychiatric disorder: 1. Yes 2. No

4. **Family history of psychiatric disorder:** 1. Yes 2. No

ANTIPSYCHOTICS

	At baseline						
	Group 1: Control	**Group 2: Risperidone**	**Group 3: Olanzapine**	**Group 4: Quetiapine**	**Group 5: Vitamin D + Vitamin E + Risperidone**	**Group 6: Vitamin D + Vitamin E + Olanzapine**	**Group 7: Vitamin D + Vitamin E + Quetiapine**

	At 2 months						
	Group 1: Control	**Group 2: Risperidone**	**Group 3: Olanzapine**	**Group 4: Quetiapine**	**Group 5: Vitamin D + Vitamin E + Risperidone**	**Group 6: Vitamin D + Vitamin E + Olanzapine**	**Group 7: Vitamin D + Vitamin E + Quetiapine**

LIPID PROFILE

	At baseline	**At 2 months**
Total cholesterol	_____	_____
LDL	_____	_____
HDL	_____	_____
Triglyceride	_____	_____

Glutathione

	At baseline	**At 2 months**
TNF alpha	_____	
Interferon gamma through PCR	_____	_____

Weight:

Gained	Not weight gained
Improved	Not improved

Brief Psychiatric Rating Scale (BPRS).

Positive and negative syndrome rating scale (PANSS)

Extra pyramidal symptom rating scale (EPSRS)

Quality of life questionnaire (WHO QOL-BREF)

INFORMED CONSENT FORM (ENGLISH)

Reg. No: _____

I, Mr./Ms./Mrs._____do hereby state willingly that I have been briefly explained about the details of this clinical research project entitled as "**Synergistic effect of Vitamin E and Vitamin D in reducing risk of side effects associated typical anti psychotics and improvement of psychiatric illness"**, which is being undertaken by **Dr.Mohammad Abid.** I do hereby give my written consent to get myself registered in this study after considering the potential benefits that it will offer to the health care services for the general public without any monetary settlements.

Participant's Signature: _____

Date: _____

Researcher's Signature: _____

Date: _____

INFORMED CONSENT FORM (URDU)

راضی نامہ

تحقیقی کام میں شرکت کرنے کے لئے آپکا بہت شکریہ اگر آپ اس کام میں دلچسپی رکھتے ہیں تو آپکو 5 ملی لیٹر خون کا نمونہ لینے کو کہا جائیگا جو کہ ایکسل کار ٹیکنیشن سرنج کے ذریعے لے گا۔ یہ نمونہ لیبارٹری میں محفوظ کیا جائیگا۔ خون کا نمونے سے کسی قسم کا کوئی ذاتی نقصان نہیں ہوگا ۔ بعد ازاں یہ نمونہ تجزیہ کے لئے اسٹاف کیا جائے گا۔ یہ تحقیقاتی کام کسی کاروباری مقصد کے لئے آپکی شناخت آپکی مرضی کے بغیر اور آپ کسی بھی وقت اس تحقیق میں آپ سے حاصل ہونے والی تمام معلومات کو حاصل کرنے کا اختیار رکھتے ہیں۔ یہ معلومات صرف تحقیق کرنے والوں کو میسر تحقیق سے لگ ہوسکتے راز میں رکھا جائے گا اور

ہونگی اگر اس تحقیق کے نتائج سے صحت کے متعلق نئی سفارشی معلومات حاصل ہوئیں تو وہ آپکے نام کو ظاہر کئے بغیر سفارشی جرنلے تا رسالے میں شائع کی جائیگی اس خون کے نمونے سے اس کے علاوہ کوئی کام نہیں لیا جائے گا۔

میں تصدیق کرتا /کرتی ہوں کہ میں نے اس اسٹڈیز کو سمجھ لیا ہے میں اپنی مرضی سے اپنے خون کا نمونہ اس تحقیقاتی کام میں لینے کے لئے تیار ہوں

نام مریض :..... دستخط :.....

راضی نامہ لینے والے کا نام ..... تاریخ :..

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