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Examensarbete

The role of omega-3 fatty acids in the treatment of schizophrenia through modification of membrane phospholipids

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Abstract

Ever since the emergence of the hypothesis that linked the aetiology of schizophrenia with abnormal membrane phospholipids composition, an increasing number of evidences have suggested reduced membrane polyunsaturated fatty acids in patients with schizophrenia. This has led to a conduct of several studies to evaluate the efficacy of omega-3 fatty acid supplement in the modification of membrane phospholipids and treatment of schizophrenia. The two main omega-3 fatty acid classes, EPA and DHA, play a vital role in membranes. This project work reviews omega-3 fatty acid studies and summarizes their outcomes. Eight original articles (nine studies) were reviewed. Six out of nine studies measured RBC membrane fatty acids levels and all six studies reported a significant increase in EPA after EPA supplement. Two studies reported increased DHA post omega-3 fatty acid and DHA supplement, respectively. One study observed a dose-dependent increment in DHA after EPA supplement. Improved symptoms were observed in seven studies, while one study found a worsening of symptoms in patients with low baseline PUFA. Moreover, out of the six studies that evaluated the correlation between symptom change and membrane fatty acids change, three studies observed a correlation between increased EPA and symptom improvement. One study reported an increased AA associated with improved symptoms, in contrast to another study, which found a correlation between increased AA and worsened symptoms. The conclusion from this project work is that EPA supplement can increase the EPA levels in membranes; however, its therapeutic effect in schizophrenia requires further investigation using larger studies.

Sammanfattning

Ända sedan tillkomsten av hypotesen som länkade etiologin av schizofreni med onormala sammansättningar av membranfosfolipider, har bevis för nedsatt membranfettsyror hos patienter med schizofreni ökat. Detta har lett till genomförandet av flera studier för att utvärdera effekten av omega-3 supplement i modifieringen av membranfosfolipider och i behandling av schizofreni. De två viktigaste omega-3 klasserna, EPA och DHA, spelar en viktig roll i membran. Detta projektarbete granskar de omega-3 studierna och sammanfattar deras resultat. Åtta originalartiklar (nio studier) granskades. Sex av nio studier mätte nivåer av RBC membranfettsyror och alla sex studierna rapporterade en signifikant ökning av EPA efter EPA behandling. Två studier rapporterade ökad DHA efter omega-3 och DHA behandling, respektive. En studie observerade en dosberoende ökning i DHA efter EPA behandling. Förbättrade symtom observerades i sju studier, medan en studie fann en försämring av symtom hos patienter med låg baseline PUFA. Av de sex studier som utvärderade sambandet mellan symtomförändring och förändring i membranfettsyror, hittade två studier samband mellan ökad EPA och symtomförbättring. En studie rapporterade en ökad AA i samband med förbättrade symtom, i motsats till en annan studie, som fann ett samband mellan ökad AA och försämrade symtom. Slutsatsen från detta projektarbete är att EPA tillägg ökar nivåer av EPA i membranfosfolipider; men dess terapeutiska effekt vid schizofreni kräver ytterligare utredning med hjälp av större studier.

Keywords

Schizophrenia, omega-3, membrane lipids, PUFA, polyunsaturated fatty acids, EPA, DHA, Eicosapentaenoic Acid, Docosahexaenoic Acid, typical antipsychotics, atypical antipsychotics

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1. INTRODUCTION

Schizophrenia is a devastating mental illness characterized by delusion, hallucination and emotional disturbance among others[1]. Its lifetime prevalence is 0.48% in average. Although it has been more than ten decades since schizophrenia has received its modern definition, its exact pathological mechanism is still not fully understood, and remains a puzzle for researchers. Several genetic and environmental factors have been believed to increase the risk of the disease. This includes ventricle enlargement, abnormal grey matter[2] and white matter, abnormal dopaminergic and glutamatergic neurons as well as pregnancy complication, a dysfunctional childhood, and some illnesses including infection and autoimmune diseases[3].

In recent years, an increasing research data has been suggesting abnormal membrane phospholipids composition as a possible indication of schizophrenia. A significant decrease in essential polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA) and arachidonic acid (AA), along with increased lipid peroxides, were found in one study with schizophrenic patients, which suggested an increased oxidative stress[4]. Similarly, a decrease in omega-3 and omega-6 fatty acids in the inner leaflet and increased phosphatidylethanolamine (PE) in the outer leaflet were observed in another study[5]. A post mortem study revealed a declined level of the phospholipids, phosphatidylcholine (PC), sphingomyelin (SM) and galactocerebrosides 1 and 2 (GC); and an increased level of phosphatidylserine (PS) in left thalamus of schizophrenics[6]. Increased release of AA and abnormal activity of phospholipase A_2 (PLA_2) have also been the focus of many researchers. In Ross et al., 1997 study, a 49% increase in calcium-independent PLA_2 activity was found in schizophrenics[7]. Moreover, other studies found a distinct correlation between severity of the illness and level of membrane lipids[8][9].

Since the emergence of these evidences, several studies have evaluated the effect of omega-3 fatty acids supplement in the treatment of schizophrenia. Cell membranes, particularly in neurons, are rich in omega-3 fatty acids and their function is enormously influenced by it. Thus, the amount of omega-3 fatty acids consumed in diet has an impact on the character of the membrane phospholipids[4]. The health benefits of omega-3 fatty acids have already been recognized for a long time. Among the documented health benefits, it includes prevention of cognitive impairment, depression, dementia, Alzheimer's disease and boosting of brain function as well as prevention of cardiovascular disease and treatment of rheumatoid arthritis[10].

2. PURPOSE

Research in recent years suggest that abnormal membrane phospholipids composition may be associated with the pathogenesis of schizophrenia, and using omega-3 fatty acids may correct this. However, evidence is limited and the results are not consistent. The purpose of this project work was to review available clinical research data and articles related to the subject and to determine the potential benefits of omega-3 fatty acids in modifying the phospholipid composition and in treatment of schizophrenia.

3. BACKGROUND

3.1. Schizophrenia

Schizophrenia is a serious mental disorder that interferes with normal behaviour and function of brain. It typically results in a variety of symptoms such as delusion and hallucination as well as cognitive and motivation decline[1].

3.1.1. Prevalence and Incidence

It is generally believed that schizophrenia affects 1% of the general population, and is distributed rather equally among genders, racial groups, cultures and countries[1]. However, a recent epidemiological study found a significant difference in lifetime prevalence based on diagnostic criteria. According to this study, the lifetime prevalence can go as high as 0.66% if the wider diagnostic criteria, which uses the broader definition of psychotic disorders, are applied. On the other hand, if more narrowed diagnostic criteria is applied, the lifetime prevalence rate can go as low as 0.30%. Such narrowed diagnostic criteria include at least 6 months of illness, onset of disease should be prior to 45 years old and negative symptoms are emphasized. A similar difference is also noted in incidence rate, which can vary from 10.2 to 22.0 per 100,000 people per year if narrowed vs. wider criteria is used, respectively. Moreover, the negative symptoms, such as emotional withdrawal, are more prominent in males than in females and due to this, the narrowed diagnostic criteria is said to favour the suggestion that the disease is more prevalent among male population than female population[2].

In addition to this, an incidence variance is also observed in sub-groups. For example, among people with high risk of schizophrenia, the incidence rate can vary from approx. 2% in third-degree relatives (e.g. if great grandparents or first cousins are affected) to approximately 50%, if a monozygotic twin carries the disease. Another area of variance in incidence is based on socio-economics, urbanism and migrant status,

in which schizophrenia is found to be more prevalent among those with low socio-economics, citified and certain immigrants than those with high socio-economics, living in rural area and natives[1].

3.1.2. Signs, Symptoms and Onset

The high-level classification of schizophrenia symptoms are the so-called, positive symptoms, negative symptoms and cognitive symptoms. The positive symptoms manifest as delusion (false belief or perception, e.g. paranoia, the thought of others being out to hurt them) and hallucination (e.g. hearing voices). They are called “positive” because the feelings or behaviours were not present in the individuals before they were sick and are considered as additions to their existing mental state. The positive symptoms are said not to be associated with neurocognitive changes[2].

The negative symptoms, which are linked to neurocognitive changes, constitute the main element of schizophrenia and influence the individuals’ ability to cope with their everyday activities. The symptoms are associated with the absence of emotion and motivation. The word “negative” here refers to the diminishing and/or loss of some behaviours or emotions that the individual had once before the illness. Individuals with negative symptoms may lack passion for everyday life and are less inclined to associate with other people. Their emotional responses can be conflicting; for example, laughing in sad situations, crying when hearing good news, or displaying no emotional reaction at all. Another manifestation of the negative symptoms are disrupted speech, lack of interest for conversation or delayed response in conversation. The individual is less likely to engage in small talks or spontaneous speech[2].

The cognitive symptoms, which are also associated with neurocognitive changes, manifest as memory and attention deficit and executive function impairment. The individual suffers from lack of focus and has difficulties in planning, organizing and memorizing. Learning and flexible thinking become a challenge. In addition to this, individuals with schizophrenia may go through mood swings; from being in a state of depression to mania or high, which is similar to bipolar disease[2].

The positive and negative symptoms do not develop simultaneously; for example, while the negative symptoms get worse over time, the positive symptoms may remain the same, or vice versa. The degree of the symptoms vary from individual to individual and from place to place[2]. The first sign of schizophrenia symptoms typically appear during teenage years and early adulthood although the prodromal symptoms/warning (such as change in cognitive functioning and social withdrawal) may occur earlier[3].

3.1.3. Causality

Due to its complexity and diversity, the true cause and underlying mechanisms of schizophrenia are not yet fully identified. However, several studies have proposed various hypotheses about the genetic and environmental risk factors that may contribute to the emergence of the disease[3].

Environmental risk factors

Several environmental risk factors have been associated with schizophrenia. For example, if a mother suffered from stress, infections, dietary deficits, pregnancy complication or poor growth of unborn baby during pregnancy, it is thought to cause neurodevelopmental impairment, which in return, may increase the child's risk to schizophrenia. A dysfunctional childhood, having an old father or having very young parents, immigration and being born in late winter or early spring have all been associated with schizophrenia risk factors. Some illnesses such as epilepsy, infection, autoimmune diseases and head injuries have also been considered to increase the risk. Furthermore, several studies have linked cannabis use to increased risk for schizophrenia[3].

Genetic risk factors

Brain image studies have suggested structural changes in various brain regions[3]. For example, a grey matter decrease, ventricle enlargement[2] and abnormal white matter have been observed in schizophrenic patients. There is also an implication that as the disease progresses, grey matter continues to decrease, specifically in temporal lobe. The prefrontal cortex of the brain, which is responsible for executive functioning, has been linked to the cognitive symptoms in schizophrenia[3].

The neurotransmitter hypothesis points towards the impairment of dopaminergic and glutamatergic neurotransmission[3]. Several studies have shown that there is an increase in the production, release and resting-state concentration of dopamine in schizophrenia. This notion is supported by the fact that all current antipsychotic medications work by blocking the dopamine receptors[2]. The dopaminergic disruption has been believed to cause the positive symptoms such as delusions and hallucinations. The glutamatergic disruption has been associated with the negative symptoms and cognitive symptoms such as emotional withdrawal and impairment of working memory[3].

Growing evidence suggest that abnormal composition of membrane phospholipids can contribute to a possible physiological mechanism of schizophrenia. This topic will be discussed in a separate section.

3.1.4. Diagnosis

Schizophrenia is diagnosed by examining the clinical symptoms of the individual using the Diagnostic and Statistical Manual of Mental Disorders (DSM) and WHO International Classification of Disease (ICD)[3]. According to the fifth edition of DSM (DSM-5), in order to be diagnosed with schizophrenia, the individual must exhibit at least two of the typical symptoms (delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behaviour and negative symptoms) for the majority of the time during 1-month period. Out of these symptoms, at least one of them should be delusions, hallucinations or disorganized speech. The symptoms must continue for at least 6 months. There should be a noticeable poor social functioning such as work and personal relationships as well as lack of self-care, compared to the individual's living standard prior to the onset of the disease. Symptoms induced by physical factors, such as alcohol, drug abuse and medications should be differentiated. Since most mental health disorders share similar symptoms, the diagnosis should distinguish schizophrenia symptoms from other psychotic disorders such as schizoaffective disorders, mood disorders and developmental disorders[11].

3.1.5. Prognosis

The long-term outcome of schizophrenia varies greatly from individual to individual. In general, up to 50% of patients with schizophrenia can have a positive outcome and lead a productive life if they get continuous support from relatives and with proper medications and counselling. However, it is noted that schizophrenic patients are expected to live 10 to 20 years shorter[3], which means 2 - 3 times higher mortality risk than that of the average population. While 40% of those deaths are caused by suicide and accidents, but 60% have natural cause – cardiovascular disease being the primary cause. Schizophrenic patients tend to have a low quality of life (such as poor diet and lack of exercise), which is induced by the negative symptoms, depression, antipsychotic drugs side effects or a number of other reasons. As a result, they are susceptible to metabolic diseases such as hypertension, obesity, diabetes and high cholesterol, which all may lead to the development of cardiovascular disease. Substance use such as alcohol, drugs and nicotine also contributes to the cardiovascular complications[12].

3.1.6. Current Treatments

Current schizophrenia treatment includes antipsychotic drugs and psychosocial therapies[3]. All antipsychotic drugs work by binding to multiple receptors but their effectiveness depend mainly on the blockage of the Dopamine D_2 receptors in the brain's dopamine pathway. There are two classes of

antipsychotic drugs: typical antipsychotics, which are also known as “conventional” or “first-generation” and atypical antipsychotics, which are also known as “second-generation”. The distinction between the two classes are made based on their binding affinity to receptors, clinical efficacy and adverse effects[13]. Both classes of drugs are known to treat positive symptoms relatively well in most cases. However, they are less beneficial for treatment of negative symptoms and cognitive symptoms,[3] even if some atypical antipsychotics have shown efficacy in this area[13]. Treating both negative and cognitive symptoms are central for the patients’ ability to function in the society[3].

Like many other drugs, antipsychotic drugs have adverse effects. The adverse effects include movement disorders, otherwise known as “extrapyramidal symptoms”, such as parkinsonism (e.g. rigidity, tremor), akathisia (motor restlessness), dystonia (muscle spasm) and tardive dyskinesia (involuntary body movement); metabolic disorders (e.g. weight gain, hyperglycaemia and high cholesterol), cardiovascular disorders, elevated plasma prolactin, sexual malfunction and sedation. Moreover, anticholinergic effects such as dry mouth, urinary retention, constipation and declined cognitive function have been reported[14]. Although the adverse effects are dependent on dosage and patients’ characteristics, atypical antipsychotics are usually associated with less extrapyramidal side effects than typical; but they have more cardiometabolic risk than typical antipsychotics[3]. Treatment compliance is usually threatened by the side effects associated with antipsychotics that leads to relapse[3][14].

In addition to antipsychotic drugs, a number of psychosocial therapies are available to aid with medication compliance and to improve social, psychological and occupational skills, which most schizophrenic patients lack. Such therapies include rehabilitation, cognitive behavioural therapy and continuous support from social circles[3].

3.2. Membrane Phospholipids

Lipids are generally defined as naturally occurring organic molecules that are primarily used as energy reserve (specifically in triglycerides form), as structural components of cell membranes and cell signalling. The cell membrane primarily contains two types of lipids: phospholipids and sterol (cholesterol). Phospholipids form the basic structure of the cell membranes; while cholesterol maintains the integrity of the membrane. The most common class of phospholipids are glycerophospholipids, which are found in cell membranes and neural tissues[15].

Phospholipids are amphipathic molecules (which means, they have both hydrophilic and hydrophobic properties)[15][16]. In aquatic environment, phospholipids position themselves so that the hydrophobic parts gather together pointing towards each other and facing away from the water molecules; while the hydrophilic parts point towards the water molecules. This arrangement allows phospholipids to naturally form a membrane bilayer in aquatic environment. Phospholipids are mainly produced in endoplasmic reticulum (ER) and distributed to organelles and cells locally. The composition and type of phospholipids vary from one organelle and cell to the other[16].

In determining the function of lipids, their physical properties are as important as their chemical properties. As membrane bilayers, phospholipids border each cell and intracellular organelles providing permeability barrier; they regulate what enters and exits the cell; and promote membrane fluidity. They protect and maintain the contents of the cell by isolating the intracellular components from the extracellular environment. They facilitate vesicle budding and fusion, thereby enabling intracellular transportation and cell division. They are involved in signalling (as first and second messengers) and recognition process. For example, arachidonic acid is precursor to the signalling substances, eicosanoids (such as prostaglandins) and cannabinoids; phosphoinositides regulate vesicular trafficking. Phospholipids also facilitate grouping of certain membrane proteins[16]. Moreover, they integrate components such as proteins that are significant for cell signalling, cell recognition and transportation[17].

3.3. Brain Membrane Phospholipids

In brain tissues, lipids represent approximately 25% of the dry weight. They are mainly found in the neuronal membranes and myelin sheaths. They serve as structural membrane components to neurons, glia cells and their organelles and they maintain fluidity[18]. They attach marker proteins, which enable unique identification of the cells[19]. Brain membrane phospholipids are precursors to specific lipid messengers and are involved in intracellular signalling (e.g. phosphoinositides), neurotransmission and they participate in proliferation, growth and protection of neurons. Neurolipids are lipids that are involved in signalling (as such, neurotransmitters) and require a specific receptor to accomplish their action. Neurolipids are synthesised on as needed basis when phospholipids are metabolized with the help of enzymes, particularly phospholipases. Neurolipid receptors include cannabinoid receptors (CB), G-protein coupled receptors (e.g. GPR55), lysophosphatidic acid (LPA) receptors and sphingomyelin (SM) receptors[18].

3.4. Change in Membrane Phospholipids Composition

Alterations of membrane phospholipids and fatty acids composition can occur for various reasons. Such modifications can interfere with the normal cellular functions. Although different cells react differently to the structural changes, it has been noted that such modifications can lead to disruption of membrane fluidity, receptor functions, signalling and transportation process as well as cell growth and synthesis of eicosanoids. The cause of cellular function disruption can be a direct result of change in lipid fluidity and domains. The other possibility is that the structure of certain proteins (e.g. enzymes, receptors and transporters) that are embedded in the membrane lipids can be sensitive to lipid structural changes and thereby their functions will be affected[17].

3.5. Fatty Acids

Fatty acids (Fig. 1) are major components of membrane phospholipids. They are amphipathic molecules that comprise a hydrophilic carboxylic acid group (-COOH) attached to a hydrophobic long straight hydrocarbon chain, in which carbon is usually an even number. If the hydrocarbon chain contains one or more double bonds between two carbon atoms, it is referred as unsaturated fatty acid ($C_nH_{(2n-1)}COOH$). If it does not contain double bonds, it is referred as saturated fatty acids ($C_nH_{(2n+1)}COOH$). The unsaturated fatty acid is further divided into two classes: mono-unsaturated fatty acids (MUFAs), in which only one double bond is present; and poly-unsaturated fatty acids (PUFAs), in which two or more double bonds are present[15].

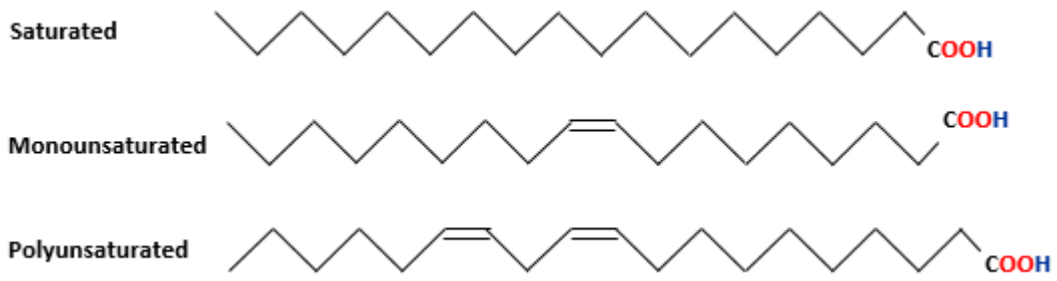

Figure 1. *The figure shows the basic chemical structure of saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid. Modified from "Omega-3 fatty acids and health" by Nettleton JA, 1995[21]*

Moreover, the position of the first double bond counting from the fatty acid end provides an additional classification to the unsaturated fatty acids group. The most common ones are omega-3 (n-3) and omega-6 (n-6) fatty acids. Omega-3 (n-3) indicates that the first double bond begins at the third carbon atom when counting from the methyl end of the fatty acid. Similarly, omega-6 (n-6) indicates that the first double bond begins at the sixth carbon atom when counting from the methyl end of the fatty acid[19]. Fatty acids fluidity is closely related to their degree of unsaturation. The higher unsaturated the fatty acids are the lower the melting point which means increased fluidity[10].

In cell membrane, fatty acids serve as structural components and precursor to a number of metabolic pathways[20][21]. They are considered as crucial for the integrity and function of brain[22]. Some of the fatty acids that are significant for biological functions include, the omega-3 fatty acids: eicosapentaenoic acid (EPA, 20:5), docosahexaenoic acid (DHA, 22:6)[15] and alpha-linolenic acid (ALA, 18:3). The omega-6 fatty acids include: linoleic acid (LA, 18:2) and arachidonic acid (AA, 20:4)[10].

3.6. Omega-3 (and Omega-6) Fatty Acids

The three most common omega-3 fatty acids are docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA)[21]. ALA is an essential fatty acid and a precursor to EPA; while EPA, in return, is a precursor to DHA. Conversion of ALA to EPA and DHA occurs through elongation, desaturation and beta-oxidation process (Fig. 2). ALA is essential because it cannot be synthesized in human body and must be obtained from food or supplements. Moreover, synthesis of EPA and DHA from ALA is not efficient and due to this, most EPA and DHA generally comes from dietary intake[10]. The main sources of ALA are vegetable oils from soybean, rapeseed and nuts. EPA and DHA are mostly found in sea foods, such as fish[21]. The omega-6 linoleic acid (LA) is another essential fatty acid, which serves as a precursor to the metabolically important omega-6 series including arachidonic acid (AA). In return, AA is a substrate for a wide range of metabolites such as eicosanoids (prostaglandins, leukotrienes and thromboxanes)[10]. The main source of LA includes vegetable oils and animal tissues[21].

For normal cell function, there need to be a balance between the level of omega-3 and omega-6 fatty acids in the body. A number of reasons have been proposed for this. Firstly, the two essential fatty acids (ALA and LA) compete for the same enzyme called delta-6-desaturase to convert their respective precursors to their corresponding DHA/EPA and the omega-6 series. Even if the enzyme naturally favours conversion of ALA over conversion of LA; however, in case of higher intake of omega-6 leading to increased plasma LA

level, the activity of the enzyme shifts towards LA conversion. This shift causes an imbalance between the synthesis of DHA/EPA and omega-6 series and result in less synthesis of DHA/EPA. Secondly, studies have shown that there may be an inadequate conversion of ALA to DHA compared to conversion of ALA to EPA in the human body, which suggests that intake of only ALA may lead to DHA deficiency. Moreover, it is noted that omega-3 series cannot be converted to omega-6 series and vice versa, thus intake of one cannot substitute the other[10].

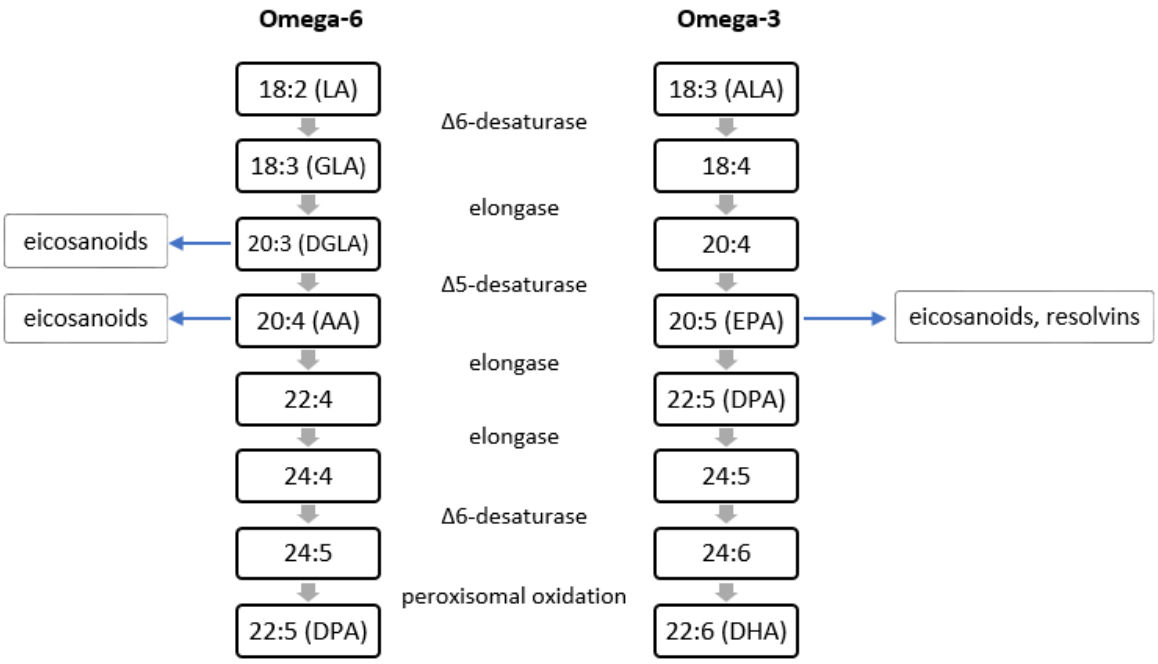

Figure 2. *The figure shows the metabolic pathway of Omega-6 and Omega-3 fatty acids. LA = linoleic acid. GLA = gamma linolenic acid. DGLA = dihomo-gamma-linolenic acid. AA = arachidonic acid. ALA = alfa-linolenic acid. EPA = eicosapentaenoic acid. DPA = docosapentaenoic acid. Modified from "Distribution, interconversion, and dose response of n− 3 fatty acids in humans" by Arterburn LM, et. al, 2006[20]*

In addition to this, DHA and EPA are not utilized similarly across different phospholipids and tissues. For example, in red blood cells (RBC), the two phospholipids, phosphatidylethanolamine (PE) and phosphatidylcholine (PC), use EPA differently, in which PE uses EPA more than PC does. DHA is a primary membrane component, while EPA is involved in eicosanoid synthesis. Moreover, blood cells prefer EPA to DHA, whereas brain tissues prefer and accumulates DHA. Additionally, neurons convert ALA and EPA to DHA; while liver converts DHA to EPA[21].

Some of the health benefits of omega-3 fatty acids include enhancing brain development[22]; reducing cardiovascular risk; treating rheumatoid arthritis symptoms through modulation of the immune system; improving brain functions; and prevention of cognitive impairment, depression, dementia and Alzheimer's disease in elderly subjects. Several studies have suggested a correlation between low level of omega-3 fatty acids and brain malfunction[10]. Significant evidences are also emerging that supports the use of omega-3 fatty acids in the treatment of schizophrenia[22].

Various mechanisms have been suggested for the effect of omega-3 and omega-6 fatty acids. Since the membrane fluidity is affected by the saturation of fatty acids in the membrane lipids, omega-3 and omega-6, which are highly unsaturated fatty acids, can decrease the cholesterol level in membrane[22][23], thereby increase fluidity that promotes normal cell function including facilitating binding of signal molecules[22][23][24]. Polyunsaturated fatty acids are an easy target for oxidation caused by free radicals (process known as lipid peroxidation) resulting in damaged membrane lipid products (known as lipid peroxides). DHA and EPA are believed to reduce lipid peroxidation. The essential fatty acids also have impact in altering the production of prostaglandin and modulation of gene expression[22]. Moreover, the membrane phospholipids, mostly in brain, are enriched with omega-3 fatty acids (particularly DHA) along with the omega-6 series (AA). This means that the accessibility of these essential fatty acids in diet have an impact on the quality and quantity of the membrane phospholipids[4].

3.7. Membrane phospholipids in schizophrenic patients

Abnormality in membrane phospholipid composition has been linked to schizophrenia by several researchers. Khan et al., 2002[4] found a significant decrease in EPUFAs (especially AA and DHA) and an increased level of lipid peroxides (thiobarbituric acid reactive substances (TBARS)), in drug-naïve first episode patients. EPUFAs were relatively lower and TBARS were higher in medicated subjects, which suggested that antipsychotics might increase level of AA and DHA. This data suggested an increased lipid peroxidation due to oxidative stress (imbalance between free radicals and antioxidants) caused by illness or treatment[4]. Nuss et al., 2009[5] found a significant increase of the phospholipid class, phosphatidylethanolamine (PE), in the outer leaflet of the RBC membrane of medicated schizophrenics. They also found a significant decrease in omega-3 and omega-6 fatty acids in the inner leaflet of the membrane. Although the exact mechanism for this was unknown, they hypothesized that an alteration of activities in enzymes responsible for phospholipids metabolism (in particular, calcium-independent PLA_2 hyperactivity) or transporters responsible for transport of phospholipids across cell membranes might

explain the membrane composition disturbance[5]. A post mortem study found a significant decrease in phosphatidylcholine (PC), sphingomyelin (SM) and galactocerebrosides 1 and 2 (GC); and an increased level of phosphatidylserine (PS) in the left thalamus of schizophrenics. SM and GC are prominent phospholipids in myelin. This data suggested that decreased oligodendrocytes (the myelinating cells) and neuronal myelination might be a possible indication of schizophrenia[6].

PLA_2 is an enzyme that hydrolyses fatty acids bond in membrane phospholipids to release AA. Hyperactivity of PLA_2 has been suggested as a possible cause of membrane disturbance in schizophrenics by researchers. Ross et al., 1997[7] demonstrated that calcium-independent PLA_2 activity in serum was significantly high with 49% increase in fluorometric assay for schizophrenics group compared to control[7]. Similarly, Ponizovsky et al., 2001[8] found a correlation between the amount of membrane phospholipids in RBC and negative/positive symptoms. Significant positive symptoms were correlated with increased SM and PE phospholipids. Significant negative symptoms were correlated with decreased SM and PE. This data suggested a possible hyperactive PLA_2 activity in patients with significant negative symptoms[8]. A magnetic resonance spectroscopy (MRS) study also revealed a link between drug-resistant positive symptoms and a phospholipid metabolism change in temporal lobe[9].

Furthermore, a significant decrease in omega-3 DHA and EPA (but not omega-6) in plasma PE and PC of first episode drug-naïve schizophrenics was reported by McEvoy et al., 2013[25]. This suggested a declined omega-3 fatty acids during the early progression of the disease, possibly due to an alteration of delta-5-desaturase activity (an enzyme required for PUFA synthesis), which is likely to stabilize post treatment[25].

3.8. Positive and Negative Syndrome Scale (PANSS)

Positive and Negative Syndrome Scale (PANSS) is a clinical scale developed to measure the prevalence of positive and negative symptoms in schizophrenia. The scale contains 30 items comprised of 7 positive scales, 7 negative scales and 16 general psychopathology scales[26]. For full details about the scale, refer to Appendix-A.

4. METHOD

This is a literature study. PubMed and Google Scholar databases were searched for relevant literatures published in English language. No date restriction was applied; however, in some instances where an updated version of a similar article was found, the latest article was used. The key words that were used

to search the literatures were schizophrenia, omega-3, lipids, membrane lipids, schizophrenia + membrane lipids, schizophrenia + Omega-3 (or PUFA or polyunsaturated fatty acids or EPA or Eicosapentaenoic Acid), schizophrenia + typical antipsychotics, schizophrenia + atypical antipsychotics. The filter was restricted to studies that included human population and free full text was available. This returned 147,507 articles. In total, 35 articles were selected and reviewed in this project work. For "Results", seven original articles (eight studies) that assessed the benefit of omega-3 fatty acids on schizophrenic patients, and one original article that assessed the preventive benefit of omega-3 fatty acids on ultra-high risk subjects were selected, reviewed and their outcomes were compared.

5. RESULTS

5.1. Summary of results

Nine studies that assessed the efficacy of omega-3 fatty acids in the treatment or prevention of schizophrenia have been reviewed and their results have been compared. The outcomes of the studies are summarized in the table shown below.

Table 1. Summary of studies

Study Reference	Study design/ study period	Dose Regimen / DOI	Outcome	
			Change in Membrane Phospholipids	**Change in symptoms (PANSS) and correlation**
Study No.1.a Peet et al., 2001. UK [27]	• 45 pts, randomized, double-blind, placebo controlled • Mean age: 44.2 ± 11.3 yrs (EPA); 42.0 ± 10.6 yrs (DHA) and 43.8 ± 10.8 yrs (placebo) • Indication: schizophrenia • 3 mon treatment	• EPA group: 2 g EPA per day • DHA group: unspecified gram DHA per day • Placebo: corn oil • Add-on • DOI = not specified	• EPA group: ↑↑EPA, ↑DHA, ↓AA • DHA group: ↑EPA, ↑↑DHA, ↑AA • Placebo: ↑EPA, ↑DHA, ↑AA	• EPA group had significantly improved total and positive scores compared to placebo. • EPA group had significantly improved positive scores compared to DHA group, the greatest improvement being in patients with highest baseline EPA level. • EPA group had significantly higher # of pts. with >25% improvement in total PANSS score than DHA & placebo

Table 1. Summary of studies

Study Reference	Study design/ study period	Dose Regimen / DOI	Outcome	
			Change in Membrane Phospholipids	**Change in symptoms (PANSS) and correlation**
				• Only minor improvement in negative symptoms in all three groups. • Significant correlation between positive symptoms improvement and increased EPA. • No correlation between symptoms change and level of DHA or AA.
Study No. 1.b Peet et al., 2001. India [27]	• 30 pts, randomized, double-blind, placebo controlled • Mean age: 33.4 ± 8.5 yrs (EPA); 36.7 ± 8.1 yrs (placebo) • Indication: Schizophrenia (new diagnose or relapse) • 3 mon treatment	• EPA group: 2 g EPA per day • Placebo: corn oil • Monotherapy • DOI = 5.7 ± 3.9 yrs (EPA); 7.1 ± 4.1 yrs (placebo)	Not measured	• EPA group had significantly improved total and positive scores compared to placebo. • EPA group had 8 of 14 pts with >50% positive symptoms improvement vs. 2 of 12 in placebo. • At study end, 6 pts in EPA group did not require antipsychotics due to symptoms improvement vs. all pts in placebo required antipsychotics.
Study No.2. Fenton et al., 2001. USA [28]	• 87 pts, randomized, double-blind, placebo controlled • Mean age: 40 yrs (SD=10) • Indication: schizophrenia,	• EPA group: ethyl-EPA 3 g per day • Placebo: mineral oil • Add-on • DOI = not specified	• EPA group: ↑EPA, ↓AA/EPA ratio • Placebo: NSC	• No change in PANSS score • No difference between active and placebo groups • No correlation between symptom and AA/EPA ratio

Table 1. Summary of studies

Study Reference	Study design/ study period	Dose Regimen / DOI	Outcome	
			Change in Membrane Phospholipids	**Change in symptoms (PANSS) and correlation**
	schizoaffective disorder • 16 wks treatment			
Study No.3. Peet et al., 2002. UK [29]	• 122 pts, randomized, double-blind, placebo controlled • Mean age: 38 yrs (20-60) (1 g EPA); 34 yrs (20-62) (2 g EPA); 37 yrs (20-56) (4 g EPA); 39 yrs (22-61) (placebo) • Indication: schizophrenia • 12 wks treatment	• 1 g ethyl-EPA per day • 2 g ethyl-EPA per day • 4 g ethyl-EPA per day • Placebo: liquid paraffin per day • Further grouping based on concomitant antipsychotic: clozapine vs Typical vs atypical • Add-on • DOI = not specified	• 1 g ethyl-EPA group: ↑EPA, ↓DHA (clozapine, typical), AA (NSC) • 2 g ethyl-EPA group: ↑EPA, ↑DHA (clozapine, atypical), ↑AA (clozapine) • 4 g ethyl-EPA group: ↑EPA, ↓DHA (clozapine, atypical), ↓AA • Placebo: NSC	• All ethyl-EPA groups on clozapine had significantly improved total, positive, negative and general psychopathology scores compared to placebo. • 2 g and 4 g ethyl-EPA groups on typical and atypical had improved PANSS scores but no significant difference from placebo due to placebo-effect. • Significant correlation between improved PANSS score and increased AA • No correlation between PANSS score and EPA or DHA.
Study No.4. Emsley et al., 2002. South Africa. [30]	• 40 pts, randomized, double-blind, placebo controlled • Mean age: 46.2 yrs (SD=10.6) (EPA); 43.6 yrs (SD=13.9) (placebo) • Indication: schizophrenia • 12 wks treatment	• EPA group: ethyl-EPA 3 g per day • Placebo: liquid paraffin • Add-on • DOI = 23.1 yrs (SD=8.5) (EPA); 22.2 yrs (SD=12.4) (placebo)	• See next row	• EPA group had significantly improved total and general psychopathology scores compared to placebo. • No significant difference in positive and negative scores between the groups

Table 1. Summary of studies

Study Reference	Study design/ study period	Dose Regimen / DOI	Outcome	
			Change in Membrane Phospholipids	**Change in symptoms (PANSS) and correlation**
Study No.5. Rensburg et al., 2009. South Africa.[31]	• NOTE: this is the same study as Emsley et al., 2002 but this article reports the analysis of the membrane PUFA change and correlation with symptom change on 32 out of the 40 patients	• EPA group: ethyl-EPA 3 g per day • Placebo: liquid paraffin • Add-on • DOI = 23.1 yrs (SD=8.5) (EPA); 22.2 yrs (SD=12.4) (placebo)	• EPA group: ↑↑EPA, ↑↑DPA, ↑DHA, ↑omega-6, ↓↓MUFA, ↓↓SFA • Placebo: ↑EPA, ↑DPA, ↑DHA, ↑omega-6, ↓MUFA, ↓SFA	• Improved total PANSS score was correlated with decreased MUFA and SFA • As a whole group, improved total PANSS score and negative symptoms were significantly correlated with increased EPA • Highest PUFA was correlated with greater than 20% overall symptom improvement • PUFA/SFA ratio were correlated with symptom improvement, where a desirable PUFA/SFA ratio yielded the highest symptom improvement
Study No.6. Ammingher et al., 2010. Austria.[32]	• 81 pts, randomized, double-blind, placebo controlled • Mean age: 16.8 yrs (SD=2.4) (Omega-3); 16.0 yrs (SD=1.7) (placebo) • Indication: ultra-high risk (attenuated psychotic symptoms and/or transient psychosis and/or trait plus state risk factors)	• Omega-3 group: 1.2 g (700 mg EPA + 480 mg DHA + 7.6 mg Vit-E) • Placebo: Coconut oil + 1% fish oil + Vit-E • Add-on • DOI = not applicable	• Omega-3 group: ↓↓Omega-6/Omega-3 ratio (i.e. significant increase in omega-3 compared to omega-6) • Placebo: NSC	• Omega-3 group had significantly improved total, positive, negative and general psychopathology scores compared to placebo at 12 wks, 6 and 12 mons. The highest change was in total and general psychopathology scores • No correlation between PANSS score improvement and omega-6/omega-3 ratio. *(It was however related to Global*

Table 1. Summary of studies

Study Reference	Study design/ study period	Dose Regimen / DOI	Outcome	
			Change in Membrane Phospholipids	**Change in symptoms (PANSS) and correlation**
	• 12 mon (12 wks treatment + 9 mon FU)			*Assessment of Functioning (GAF))*
Study No.7. Bentsen et al., 2013. Norway.[33]	• 99 pts, randomized, double-blind, placebo controlled • Mean age: 28.3 (SD=5.8) (double placebo); 25.7 yrs (SD=5.4) (active EPA); 27.6 yrs (SD=7.1) (double active); **28.6 yrs (SD=6.3) (active vitamin)* • Indication: schizophrenia/related psychosis • 16 wks treatment	• Double placebo group (paraffin) • Active EPA group (2 g ethyl-EPA + Placebo Vit-E/C per day) • Double active (2 g EPA + Vit-E/C per day) • **Active vitamins group (Placebo ethyl-EPA + Vit-E/C per day)* • Further grouping based on baseline PUFA: Low PUFA vs High PUFA • Add-on • DOI = 7 yrs (2-10) (double placebo); 2 yrs (1-5.5) (active EPA); 3.5 yrs (1-6.5) (double active); *3.5 *yrs (2-8) (active vitamin)*	• Active EPA and Double Active groups (as a whole – without baseline PUFA consideration): ↑EPA • Low PUFA, Active EPA group: ↑total PUFA, ↑DHA, ↑AA • High PUFA, Active EPA group: NSC • High PUFA, Double Active group: ↑AA • Low PUFA, Double Active group: NSC • Placebo: NSC	• Low PUFA, Active EPA group had significantly worsened positive score • Low PUFA, Double Placebo and Double Active groups, had no change • High PUFA, all groups had no change • Placebo had no change • Worsened positive symptoms were correlated with increased AA and total PUFA • Change in total PANSS score, Negative and General symptoms were not correlated with change in PUFA
Study No.8. Jamilian et al., 2014. Iran.[34]	• 60 pts, randomized, double-blind, placebo controlled • Mean age: 32.01 yrs (SD=7.13) (omega-3); 31.01 yrs (SD=8.81) (placebo) • Indication: schizophrenia	• Omega-3 group: 1000 mg omega-3 per day • Placebo • Add-on • DOI = 9.3 yrs (SD=5.03) (omega-3); 10.11 yrs (SD=5.24) (placebo)	• Not measured	• Both omega-3 and placebo groups had significantly improved positive, negative and general psychopathology scores • No significant difference between the groups in positive and negative scores

Table 1. Summary of studies

Study Reference	Study design/ study period	Dose Regimen / DOI	Outcome	
			Change in Membrane Phospholipids	**Change in symptoms (PANSS) and correlation**
	• 8 wks treatment			• But, significant difference between the groups in general psychopathology score after 4 wks favouring omega-3 group

Pts = patients. FU = Follow-up. DOI = duration of illness. ↑ = significant increase. ↑ = non-significant increase. ↓ = significant decrease. ↓ = non-significant decrease. NSC = no significant change. Wks = weeks. Mon = months. PANSS = Positive and Negative Syndrome Scale. Add-on = added on antipsychotics. Atypical = atypical antipsychotics. Typical = typical antipsychotics. Unspecified = unspecified type of antipsychotics. MUFA = mono unsaturated fatty acids. SFA = saturated fatty acids. *Active vitamins is irrelevant to this paper, thus not discussed.

5.2. Study No. 1a and 1b – Two double-blind placebo-controlled pilot studies of eicosapentaenoic acid in the treatment of schizophrenia (Peet et al., 2001)[27]

(a) Study No. 1.a – Double-blind placebo controlled trial comparing EPA and DHA

Methods

This was a pilot study. Schizophrenic patients (n=55) with a score of greater than 40 in PANSS were randomized to a double-blind placebo controlled pilot study in the UK. The treatment period was 3 months and they continued on their usual antipsychotics during the treatment period. Only 45 patients' data was available for analysis and out of which 15 patients were assigned to 2 g EPA per day, 16 patients were assigned to a DHA enriched oil (dosage not specified) per day and 14 patients were assigned to placebo corn oil per day. The patients' mean age was 44.2 ± 11.3 (standard deviation, SD) years in EPA, 42.0 ± 10.6 years in DHA and 43.8 ± 10.8 years in placebo group. The primary outcome of the study was to compare change in symptoms from baseline to end of treatment among EPA, DHA and placebo groups[27].

Results

At the end of the treatment, the EPA group showed a significant improvement in total PANSS score and positive symptom score compared to placebo. Moreover, the EPA group showed a significant improvement in positive symptoms compared to DHA group. The mean positive score from baseline to post-treatment changed from 18.9 (SD 5.4) to 14.6 (5.9) in EPA group; from 17.8 (5.4) to 16.7 (5.3) in DHA

group; and from 18.7 (5.7) to 15.8 (5.1) in placebo group. The mean total score from baseline to post-treatment changed from 69.9 (12.9) to 55.5 (12.2) in EPA group; from 73.4 (17.9) to 65.3 (19.0) in DHA group; and from 76.2 (20.6) to 65.9 (14.9) in placebo group. Moreover, the number of patients with more than 25% improvement in total PANSS score was significantly higher in EPA group than in both DHA and placebo groups. Only minor improvement in negative symptoms was observed for EPA, DHA and placebo groups with mean improvement percentage of 9.7%, 9.4% and 8.0%, respectively from baseline to study end. Individual mean score change was not specified for negative and general psychopathology[27].

EPA, DHA and AA levels were measured in RBC membranes. Following the treatment, the EPA and DHA levels increased significantly in EPA and DHA groups, respectively compared to placebo. The mean change from baseline to post treatment is as follows. In EPA group, EPA increased from 0.8 (0.3) to 3.4 (1.6), DHA increased from 4.6 (1.0) to 5.9 (1.6), and AA decreased from 11.5 (4.8) to 10.8 (3.3). In DHA group, EPA increased from 0.7 (0.2) to 2.2 (1.4), DHA increased from 3.8 (1.1) to 8.3 (2.0) and AA increased from 11.6 (3.8) to 11.9 (1.9). In Placebo group, EPA increased from 0.6 (0.2) to 0.8 (0.3), DHA increased from 3.7 (1.1) to 4.3 (1.7), AA increased from 10.4 (4.8) to 12.9 (5.2)[27].

There was a significant correlation between positive symptoms improvement and increased EPA levels in EPA group. The greatest positive symptoms improvement was seen in patients with highest baseline EPA level. Similar correlation was not found in DHA and placebo group[27].

(b) Study No. 1.b – Placebo-controlled trial of EPA as a sole treatment for schizophrenia

Methods

A randomized, double-blind, placebo controlled pilot trial in India, which consisted of 30 schizophrenic patients who were newly diagnosed or had a relapse incident. 21 patients received their last antipsychotics at least 2 weeks prior to entering the study and 9 patients were drug naïve. During the study period, no antipsychotics were allowed unless clinically required. The patients were assigned to 2 g per day EPA (n=15) or placebo corn oil (n=15). The mean age was 33.4 ± 8.5 years in EPA group and 36.7 ± 8.1 years in placebo group. Duration of illness was 5.7 ± 3.9 years in EPA group and 7.1 ± 4.1 years in placebo. The primary outcome of the study was to determine the requirement for initiation of antipsychotics and the length of the treatment as well as change in symptoms from baseline to study end[27].

Results

The treatment period was 3 months. At the end of the treatment, the EPA group had a significant improvement in PANSS score, specifically in positive symptoms compared to placebo. The mean score change from baseline to post-treatment was as follows. The positive score changed from 23.1 (8.7) to 12.5 (2.8) in EPA group; and from 24.7 (8.2) to 17.7 (8.6) in Placebo group. The total score changed from 70.4 (10.1) to 44.6 (8.7) in EPA group; and from 79.3 (18.6) to 57.1 (15.5) in placebo group. Individual mean score change was not specified for negative and general psychopathology. Moreover, 8 out of 14 patients in EPA and 2 out of 12 patients in placebo had greater than 50% positive symptoms improvement. Although no statistical comparison was made due to small number, there was no significant difference observed between those who had been treated on antipsychotics before entering the study and drug naïve patients. Additionally, at the end of the study, 6 patients in EPA group did not require antipsychotics, out of which, 4 never required antipsychotics throughout the treatment period; while 1 patient received antipsychotics for the first week of the treatment period, and 1 patient received a single dose of antipsychotics early in the trial, which was statistically equivalent to 14 days treatment. On the contrary, all patients in placebo group were using antipsychotics at study end. The average duration of antipsychotics usage was around 1 month in EPA group and more than 2 months in placebo[27].

Study No. 1a and 1b discussion and conclusion

In conclusion, both of the pilot studies demonstrated a positive outcome of EPA supplement in reducing PANSS scores, specifically positive symptom scores compared to DHA and placebo. The advantage of EPA over DHA was unexpected provided DHA is found in large quantity in membrane phospholipids unlike EPA, which is only a minor constituent of membranes. Therefore, the study did not anticipate the EPA treatment effect to be a result of a direct incorporation of EPA into membrane phospholipids. Moreover, the first study found a correlation between increased membrane EPA levels in RBC (following EPA supplement) and positive symptoms improvement. This suggested that those patients with lowest EPA and lowest treatment response might have a severe metabolic abnormality, which required more intervention than EPA supplement. The study concluded stating that the result from these studies support the phospholipid hypothesis of schizophrenia. If larger studies could confirm the efficacy of EPA, it could be a desirable treatment due to its favourable side effects and other health benefits[27].

5.3. Study No. 2 – A Placebo-Controlled Trial of Omega-3 Fatty Acid (Ethyl Eicosapentaenoic Acid) Supplementation for Residual Symptoms and Cognitive Impairment in Schizophrenia (Fenton et al., 2001)[28]

Methods

A 16-week, randomized, double-blind, placebo controlled study on 87 schizophrenic patients aged 18 to 65 (mean age 40 years (SD=10)) with residual symptoms, i.e. incomplete remission despite antipsychotics. As part of the inclusion criteria, residual symptoms were defined as patients with a PANSS score of >4 in at least one positive and/or one negative symptom, or a total score of >45 out of which 3 scores or more being in at least 3 positive or negative symptoms. 70% of the patients had schizophrenia and 30% had schizoaffective disorder. The mean age of first diagnosis was 20.8 years (SD=6.3). 22% were on 2 unspecified neuroleptics, 39% were on risperidone, olanzapine, or quetiapine and 28% were on clozapine and 1 patient was not on antipsychotics. The mean consumption of dietary omega-3 fatty acids was 367 mg/day (SD=378) and had no significant difference between the groups. The primary and secondary outcomes included change in symptoms and correlation between symptom change and AA/RBC ratio[28].

Results

The patients were assigned to 3 g ethyl-EPA per day (n=43) or placebo, mineral oil (n=44). At the end of the treatment, no notable change in PANSS score was observed in both EPA and placebo groups. The baseline to post-treatment mean total PANSS score changes were as follows:

- In EPA group: from baseline 74 (SD=16) to 69 (SD=18) at Week 12 and to 69 (SD=16) at Week 16
- In placebo: from baseline 76 (SD=18) to 69 (SD=17) at Week 12 and to 70 (SD=18) at Week 16

The mean RBC membrane AA/EPA ratio change from baseline to Week 16 was measured and EPA group had -29.4 (SD=11.3) and placebo group had -4.0 (SD=12.2). This indicated that the EPA level in RBC membranes increased in EPA group. The AA/EPA ratio did not have any significant correlation with the symptom outcome[28].

Study No. 2 discussion and conclusion

The study concluded that ethyl EPA supplement was not found to be effective for treatment of extensive symptoms in such population who had been ill for a long time. However, they pointed out that it might

produce a different outcome if tested in population with less severe symptoms, less duration of illness, different dosage and/or duration of treatment[28].

5.4. Study No. 3 – A Dose-Ranging Exploratory Study of the Effects of Ethyl-Eicosapentaenoic in Patients with Persistent Schizophrenic Symptoms (Peet et al., 2002)[29]

Methods

A randomized, double-blind and placebo controlled study included 122 schizophrenic patients, out of which 115 completed post-baseline assessment. The study was conducted at 9 sites in the UK. The patients had ≥ 50 in total PANSS score and ≥ 15 in positive symptom score, and they had their initial diagnoses earliest 20 years prior to inclusion. They stayed on their standard medications throughout the study. 31 patients were taking clozapine; 48 were taking olanzapine, risperidone or quetiapine; and 36 were receiving typical antipsychotics. The primary outcome of the study was change in total PANSS score from baseline to end of treatment. Moreover, fatty acids were measured in RBC membrane[29].

The patients were assigned to 4 treatment groups: 1 g ethyl-EPA (n=29), 2 g ethyl-EPA (n=28), 4 g ethyl-EPA (n=27) or liquid paraffin (placebo, n=31)) per day. The patients' mean age was 38 years (20-60) in 1 g EPA group, 34 years (20-62) in 2 g EPA group, 37 years (20-56) in 4 g EPA group and 39 years (22-61) in placebo group. The treatment period was 12 weeks. The results were adjusted to the three classes of antipsychotics that patients were on: clozapine, typical and atypical[29].

Results

At the end of the EPA treatment,

- EPA increased in all EPA groups for all antipsychotics, the highest being in 4 g ethyl-EPA group, while placebo group had no significant change. DHA increased in 2 g ethyl-EPA, clozapine group, but it decreased in 1 g ethyl-EPA, clozapine and typical groups, and in 4 g ethyl-EPA, clozapine and atypical groups. For all other groups and antipsychotics, the DHA change was insignificant because it either was a small change or was not different from placebo. AA increased significantly in 2 g ethyl-EPA, clozapine group but decreased significantly in 4 g ethyl-EPA, clozapine and atypical. For all other groups and antipsychotics, the AA change was insignificant because it either was a small change or was not different from placebo.
    - The actual changes in red blood cell membranes' PUFAs are presented below:

- In placebo, EPA changed by -0.6±5.0 (SD) for clozapine; by +3.7±11.4 for atypical; by +4.8±12.6 for typical antipsychotics groups. DHA changed by -0.5±20.0 for clozapine; by +13.7±30.0 for atypical; by +5.4±17.3 for typical antipsychotics groups. AA changed by -12.6±63.1 for clozapine; +29.6±50.3 for atypical, +12.4±66.4 for typical antipsychotics groups.
- In 1 g EPA, EPA changed by +2.4±31.3 for clozapine; by +14.6±16.2 for atypical; by +16.7±12.4 for typical antipsychotics groups. DHA changed by -2.2±35.5 for clozapine; by +4.3±18.1 for atypical; by -1.4±11.7 for typical antipsychotics groups. AA changed by +2.7±73.9 for clozapine; +15.9±42.1 for atypical; by -10.6±35.8 for typical antipsychotics groups.
- In 2 g EPA, EPA changed by +32.7±26.7 for clozapine; by +31.5±21.6 for atypical; by +23.9±19.4 for typical antipsychotics groups. DHA changed by +12.2±26.7 for clozapine; by +11.4±14.1 for atypical; by +2.3±17.5 for typical antipsychotics groups.
- In 4 g EPA, EPA changed by +49.0±42.0 for clozapine; by +34.9±36.0 for atypical; by +38.9±28.9 for typical antipsychotics groups. DHA changed by -4.8±25.6 for clozapine; by -9.1±23.6 for atypical; by +6.0±23.5 for typical antipsychotics groups. AA changed by -26.5±75.1 for clozapine; by -36.1±60.2 for atypical; by -0.7±63.7 for typical antipsychotics groups.

- Additionally, all ethyl-EPA groups on clozapine showed a significant decrease in total, positive, negative and general psychopathology PANSS scores compared to placebo. The highest decrease was in 2 g ethyl-EPA group. Patients in 2 g and 4 g ethyl-EPA groups (typical and atypical) had improvement on PANSS scales, but a similar placebo-effect increment was shown in the placebo group, and due to this, it was considered insignificant.
  - The mean PANSS score for patients taking **typical antipsychotics** changed from baseline to end of treatment as follows:
    - Total PANSS score changed from 75.0 to 58.4 in placebo group; from 73.0 to 63.4 in 1 g group; from 74.8 to 62.4 in 2 g group and from 80.8 to 66.7 in 4 g group.
    - Positive score changed from 20.3 to 14.6 in placebo group; from 17.6 to 14.7 in 1 g group; from 18.8 to 14.2 in 2 g group; from 22.4 to 17.2 in 4 g group.

- Negative score changed from 17.2 to 14.3 in placebo group; from 16.7 to 15.2 in 1 g group; from 20.2 to 16.0 in 2 g group and from 18.5 to 15.3 in 4 g group.
- General score changed from 37.5 to 29.6 in placebo group; from 38.8 to 33.6 in 1 g group; from 35.8 to 32.2 in 2 g group; from 39.9 to 34.2 in 4 g group.

- The mean PANSS score for patients taking **atypical antipsychotics** changed from baseline to end of treatment as follows:
  - Total PANSS score changed from 80.3 to 61.3 in placebo group; from 73.3 to 59.5 in 1 g group; from 84.7 to 64.9 in 2 g group; from 82.1 to 73.7 in 4 g group.
  - Positive score changed from 21.5 to 16.6 in placebo group; from 18.1 to 14.6 in 1 g group; from 20.7 to 15.9 in 2 g group; from 21.6 to 18.7 in 4 g group.
  - Negative score changed from 17.8 to 13.8 in placebo group; from 17.6 to 15.0 in 1 g group; from 22.0 to 16.6 in 2 g group; from 20.4 to 17.7 in 4 g group.
  - General score changed from 41.1 to 30.9 in placebo group; from 37.6 to 29.8 in 1 g group; from 42.0 to 32.3 in 2 g group; from 40.2 to 37.3 in 4 g group.
- The mean PANSS score for patients taking **clozapine** changed from baseline to end of treatment as follows:
  - Total PANSS score changed from 77.3 to 72.7 in placebo group; from 80.2 to 65.6 in 1 g group; from 86.1 to 63.7 in 2 g group; from 70.3 to 58.8 in 4 g group.
  - Positive score changed from 21.3 to 19.3 in placebo group; from 19.7 to 16.2 in 1 g group; from 20.3 to 15.0 in 2 g group; from 18.5 to 15.0 in 4 g group.
  - Negative score changed from 17.7 to 16.4 in placebo group; from 22.1 to 16.9 in 1 g group; from 22.1 to 16.9 in 2 g group; from 15.8 to 13.7 in 4 g group.
  - General score changed from 38.3 to 37.0 in placebo group; from 38.4 to 32.4 in 1 g group; from 43.7 to 31.8 in 2 g group; from 36.0 to 30.2 in 4 g group.

- There was a significant correlation between increased RBC membrane AA and decreased PANSS score; however, change in EPA and DHA had no correlation with PANSS score[29].

Study No. 3 discussion and conclusion

Ethyl-EPA in moderate dose was suggested by the study to have an inhibition effect on PLA_2 enzyme, which could lead to a reduced release of AA from membrane phospholipids and thus increased AA level in membrane. This could probably be the reason for an increase in AA in 2 g ethyl-EPA group (a group that

also showed a significant PANSS score improvement). The study also reported that the ineffectiveness of 4 g ethyl-EPA was correlated with the decrease in AA in this group as EPA in high amount could probably displace AA. In conclusion, the EPA therapeutic effect might be mediated by PLA_2 and could be related to AA levels rather than DHA and EPA levels in membrane. This is also in line with the notion about hyperactivity of PLA_2 in schizophrenia[29].

5.5. Study No. 4 – Randomized, Placebo-Controlled Study of Ethyl-Eicosapentaenoic Acid as Supplemental Treatment in Schizophrenia (Emsley et al., 2002)[30]

Methods

The study was conducted in South Africa on 40 schizophrenic patients aged 18 to 55 years who had a total PANSS score of >50 and had been on stable antipsychotics for at least 6 months prior to inclusion. The patients were randomized to either 3 g ethyl-EPA/day (n=20) or placebo, liquid paraffin/day (n=40) in a blinded fashion. The mean duration of illness was 23.1 years for EPA group and 22.2 for placebo group. The mean age was 46.2 years (SD=10.6) in EPA and 43.6 years (SD=13.9) in placebo. Both groups were on antipsychotics in parallel with the study drugs. They had a balanced meal and their usual diet contained only a small amount of EPA (0.56 – 1.13 g per week), and there was no difference in PUFA intake between the groups. The primary and secondary outcome of the study was to measure the baseline to end of treatment change in total PANSS score and individual positive, negative and general psychopathology sub-scores, respectively[30].

Results

Following 12 weeks treatment, the mean total PANSS score significantly decreased by 12.6 (SD=14.0) from baseline in EPA group compared to placebo, in which it decreased by 3.1 (SD=13.3). After adjusting the outcome to gender, duration of illness and concomitant medication, the significance stayed the same, although patients on typical antipsychotics inclined to have more reduction (mean = 17.4 (SD=12.1)) than those on clozapine (mean=6.8 (SD=14.6)). When looking at the individual PANSS sub-scales, only the general psychopathology score differed significantly for the EPA group compared to placebo. There was no significant difference in positive and negative scores between the EPA and placebo groups[30].

Study No. 4 discussion and conclusion

The conclusion from this study was that EPA could be a safe add-on treatment in chronic schizophrenia. EPA also triggered a quick response, which was confirmed by the fact that the significant reduction in symptoms was initially seen only after 3 weeks of EPA intervention. The study limiting factors were the small number of the population and that the efficacy of EPA on its own to treat schizophrenia still need to be investigated[30].

5.6. Study No. 5 – Changes in erythrocyte membrane fatty acids during a clinical trial of eicosapentaenoic acid (EPA) supplementation in schizophrenia (van Rensburg et al., 2009)[31]

Methods

This is the same study as Emsley et al., 2002[30] but this article reports the analysis of the RBC membrane PUFA change and correlation with symptom change on 32 out of the 40 patients. Emsley et al., 2002[30] reported only symptom outcome. For details of method and study design, refer to Study No. 4 (Emsley et al., 2002)[30].

Results

The patients were treated for 12 weeks. At the end of the treatment, the mean red blood cell membranes' PUFAs (in µg/100 µg) changed from baseline to end of treatment as follows:

- In EPA group, EPA changed from 0.23±0.20 (SD) to 2.97±1.69; DPA changed from 0.75±0.67 to 3.2±1.74; DHA changed from 2.65±2.19 to 3.52±2.15; AA changed from 6.15±4.68 to 7.54±3.03. The omega-9, monounsaturated fatty acids: eicosaenoic acid decreased from 0.22±0.11 to 0.12±0.04 and nervonic acid decreased from 5.23±1.18 to 4.27±1.57. The saturated fatty acids: arachidic acid decreased from 0.67±0.27 to 0.52±0.14 and lignoceric acid decreased from 8.82±2.93 to 6.7±2.43.
- In placebo group; EPA changed from 0.28±0.15 to 0.40±0.23; DPA changed from 0.82±0.79 to 1.30±0.78; DHA changed from 2.69±1.83 to 3.92±2.09; AA changed from 6.82±4.92 to 8.90±4.08. The omega-9, monounsaturated fatty acids: eicosaenoic acid increased from 0.21±0.08 to 0.23±0.17and nervonic acid decreased from 5.15±1.40 to 4.46±1.55. The saturated fatty acids: arachidic acid decreased from 0.62±0.17 0.53±0.18 and lignoceric acid decreased from 7.53±2.19 to 6.41±1.72.

The omega-3 fatty acids, EPA and its long-chained derivative, docosapentaenoic acid (DPA, 22:5), increased significantly; while DHA increased non-significantly. EPA increased by 1189.99% and DPA by 328.03%. Placebo group had a non-significant omega-3 increase. No significant change in omega-6 was observed in both groups. 13 out of 16 patients in EPA group showed a significant decrease in saturated and monounsaturated fatty acids, highest being a 49.32% decrease in the omega-9 fatty acid, eicosaenoic acid (20:1). Placebo group had non-significant reduction. It was also observed that females increased their EPA (1,553.98%), DPA (351.19%), DHA (51%) and AA (25.61%) more than males (1,123.02%, 320.91%, 27.52% and 21.63%, respectively). Moreover, the proportion of AA to EPA in the EPA group was -31.33, compared to -0.06 in the placebo group[31].

Decreased saturated and monounsaturated fatty acids were correlated with improved total PANSS score. Increased EPA was significantly correlated with improved total PANSS score and negative symptoms but not with positive symptoms and general psychopathology. 4 patients (2 men and 2 women) in the EPA group, who had the highest omega-3 increase, showed greater than 20% improvement in total PANSS score. The proportion of total PUFA to saturated fatty acids was also compared against the PANSS score in EPA group and the PANSS score appeared to decrease until a desirable proportion was achieved and then increase. This result implied that the fatty acid metabolism and incorporation among the population was different. The 4 patients with more than 20% symptom improvement had a desirable proportion of total PUFA to saturated fatty acids though[31]. Note: individual PANSS score changes were not specified in the article.

Study No. 5 discussion and conclusion

The study concluded stating that there was a correlation between fatty acid composition in RBC membrane and symptom improvement. EPA supplement increased membrane EPA and DPA significantly but the lack of DHA synthesis would require further investigation as it could probably be attributed to genetic abnormality (e.g. enlongase and desaturase enzymes abnormality) in synthesis or in incorporation of DHA into membrane phospholipids in schizophrenics[31].

5.7. Study No. 6 – Long-Chain omega-3 Fatty Acids for Indicated Prevention of Psychotic Disorders (Amminger et al., 2010)[32]

Methods

Subjects (n=81) aged 13 to 25 years with ultra-high risk for psychosis were randomized to a double-blind placebo controlled, 12-months trial conducted in Austria. The study defined ultra-high risk as the presence of attenuated positive psychotic symptoms or transient psychosis using PANSS, or patients with schizotypal personality disorder, or a history of psychotic disorder in first-degree relatives using Family History Research Diagnostic Criteria and a 30% decrease in functioning using Global Assessment of Functioning Scale (GAF). The subjects were randomized to omega-3 fatty acids (n=41) or placebo (n=40). The 1.2 g daily dose of omega-3 fatty acids contained a combination of 700 mg EPA, 480 mg DHA and 7.6 mg mixed Vitamin-E. The placebo contained coconut oil, 1% fish oil (to resemble omega-3 test) and Vitamin-E. The patients' mean age was 16.8 years (SD=2.4) in omega-3 and 16 years (SD=1.7) in placebo. The treatment period was 12 weeks followed by 9 months observational period. The primary outcome of the study was conversion to psychosis and the secondary outcome was change in PANSS, GAF (and MDRS) score. Composition of omega-3 and omega-6 fatty acids in RBC was also measured at baseline and 12 weeks follow-up. 76 patients completed the study as per protocol[32].

Results

Following a 12-month study period, 2 of 40 subjects in the omega-3 group, and 11 of 40 in the placebo group progressed to psychotics, which is a 22.6% difference between the groups. Out of the progressed subjects, 11 were to schizophrenia or schizophreniform. Omega-3 showed a significant decrease in PANSS scores and a significant improvement in GAF scores. The changes in mean PANSS and GAF scores from baseline to post treatment are presented below:

- In omega-3 group, total score at baseline 59.9 (±2.7) decreased by −15.7 (±2.8); Positive score at baseline 15.0 (±0.7) decreased by −4.4 (±0.8); negative score at baseline 14.1 (±0.9) decreased by −3.9 (±0.9); general psychopathology at baseline 30.9 (±1.4) decreased by −7.5 (±1.5). GAF score at baseline 61.0 (±2.3) decreased by 17.7 (±2.3).
- In placebo group, total score at baseline 57.2 (±2.7) decreased by −4.4 (±2.8); positive score at baseline 14.2 (±0.7) decreased by −1.5 (±0.8); negative score at baseline 13.6 (±0.9) decreased by

−0.8 (±0.9); general psychopathology at baseline 29.4 (±1.4) decreased by −2.1 (±1.5). GAF score at baseline 60.0 (±2.4) decreased by 7.2 (±2.3).

Additionally, a significant decrease in RBC omega-6/omega-3 ratio, which is an increase in omega-3 fatty acids in relation to omega-6, was observed in the omega-3 group versus placebo. Total omega-6 fatty acids at baseline was 28.8 (±2.8) in omega-3 group and 28.3 (±2.5) in placebo group. Total omega-3 fatty acids at baseline was 5.6 (1.2) in omega-3 group and 5.3 (1.0) in placebo group. Post treatment, the omega-6 to omega-3 ratio decreased by -2.0 (SD=1.2) in omega-3 group and by -0.1 (SD=0.7) in placebo group. The change in the fatty acids was correlated with general function improvement (GAF score) but not with symptom improvement score (PANSS score)[32].

Study No. 6 discussion and conclusion

Omega-3 fatty acids diminished the development of psychosis as well as improved symptoms and general functioning. Moreover, the subjects that did not progress to psychosis remained in the same status during the 9 months observational period post treatment. The study attributed the later to the neuroprotective nature of omega-3 fatty acids probably by reducing oxidative stress in membrane phospholipids, as it was previously established that EPA could increase the antioxidant "glutathione" in temporal lobe of first-episode psychosis. The study limitations were (a) ultra-high risk criteria was specific to the study; (b) age limitation that subjects older than 25 might not respond similarly; and (c) whether omega-3 fatty acids delayed or prevented the first episode was unknown[32].

5.8. Study No. 7 – Omega-3 fatty acid and vitamins E+C in schizophrenia (Bentsen et al., 2013)[33]

Methods

The Norwegian study randomized 104 patients with schizophrenia, schizoaffective or schizophreniform disorder aged 18-39 years in a multi-centred, randomized, and double-blind placebo controlled study. The median duration of illness was 7 years for double placebo group, 3.5 years for active vitamin group, 2 years for active EPA group and 3.5 years for double active group. 99 patients were assigned to four treatment arms. Double Placebo group (24 patients) received paraffin. Active Vitamins group (26 patients) received placebo EPA and Vitamin E + C, but this group will not be discussed further due to its irrelevance to this project work. Active EPA group (33 patients) received 2 g ethyl-EPA and placebo vitamins. Double Active group (16 patients) received 2 g ethyl-EPA and Vitamin E/C. The patients' mean age was 28.3 (SD=5.8) in double placebo group; 25.7 years (SD=5.4) in active EPA group; 27.6 years (SD=7.1) in double active group

and 28.6 years (SD=6.3) in active vitamin group. The patients were on their standard antipsychotic drugs during the study period. At baseline, the patients PUFA level in RBC membrane was measured for 97 patients and they were sub-categorized into low PUFA (n=30, mean PUFA = 102 µg/g) and high PUFA (n=67, mean PUFA = 442 µg/g) patients for outcome analysis. PUFA refers to sum of omega-3 and omega-6 fatty acids in RBC membrane. The primary and secondary endpoints were change in total PANSS and sub-scale PANSS scores, respectively[33].

Results

After 16 weeks of treatment, no significant change in total PANSS score was observed between the active and placebo groups. However, a difference was observed when adjusting the outcome to the baseline RBC PUFA. In low PUFA population, the total PANSS score for Active EPA group (EPA + placebo Vit) decreased from 80.8 to 71.0 compared to Double Placebo group in which total PANSS score decreased from 80.8 to 58.4. The total score changes in low PUFA were due to positive symptoms only (in particular, persecutory delusions). There were no changes observed in negative symptoms and general psychopathology subscales. The Double Active group (EPA + Vitamin E/C) in low PUFA population did not show a significant difference in PANSS score compared to placebo. In high PUFA population, EPA alone and combination of EPA + Vitamin E/C did not have any effect on the PANSS score[33]. Note: a list of PANSS scores was provided only as graph rather than list.

In relation to RBC membrane PUFA, in general, EPA supplement increased membrane EPA (+22.5 µg/g). When considering baseline PUFAs, EPA alone supplementation increased total PUFA, DHA and AA in low PUFA group significantly; but similar change was not seen in the high PUFA group. Supplement of EPA + Vitamin E/C did not lead to any significant change in low PUFA patient; however, it decreased the AA (−41.3 µg/g) in high PUFA patient. Positive symptoms and AA were positively correlated. Change in total PANSS score, Negative and General symptoms were not correlated with change in PUFA[33]. Note: for membrane PUFA levels, only estimated changes were provided but not actual changes.

Study No. 7 discussion and conclusion

The study concluded that adding a combination of EPA + Vitamin E/C to current antipsychotics is well tolerated, but not efficacious. However, adding only EPA to current antipsychotics induced positive symptoms in patients with low RBC PUFA. The probable cause for the exacerbation of the positive symptoms in low PUFA patients were hypothesized to be related to impaired redox regulation (oxidative

stress) in low PUFA patients and adding the long chained PUFA, ethyl-EPA, would intensify the lipid peroxidation. When EPA was combined with Vitamin E/C, there was a neutralization of oxidation via mutual redox regulation[33].

5.9. Study No. 8 – Omega-3 as supplemental treatment in schizophrenia (Jamilian et al., 2014)[34]

Methods

A randomized, double-blind, placebo controlled trial with 60 schizophrenic patients aged 15 – 55 conducted in Iran. 30 patients were assigned to omega-3 group and received 1000 mg omega-3 fatty acids per day (ingredients are not specified). 30 patients were assigned to a placebo treatment. The patients' mean age was 32.01 years (SD=7.13) in omega-3 group and 31.01 years (SD=8.81) in placebo group. Both groups were treated with atypical antipsychotic drugs in parallel with omega-3 fatty acids and placebo. Mean duration of illness was 9.3 years in omega-3 group and 10.11 years in control placebo group. The total treatment period was 8 weeks. Positive and Negative Syndrome Scale (PANSS) was measured at baseline and at each clinical visit[34].

Results

After 8 weeks of treatment, both omega-3 and placebo groups showed a significant decrease in PANSS score compared to baseline. There was no significant difference in positive and negative symptoms between the groups; however, the omega-3 group showed a significant decrease in general psychopathology score after 4 weeks of treatment compared to the placebo group.

- For omega-3 group, the change in mean PANSS score from baseline to week 8 were as follows: from 26.66±3.33 to 14.00±2.79 for positive symptoms, from 23.83±3.35 to 12.13±2.59 for negative symptoms, from 45.90±4.68 to 22.33±3.46 for general score and from 96.13±9.61 to 49.13±5.31 for total score.
- For placebo group, the change in mean PANSS score from baseline to week 8 were as follows: from 27.63±3.94 to 14.66±2.48 for positive symptoms, from 23.06±3.64 to 11.26±2.80 for negative symptoms, from 48.16±3.90 to 26.70±3.46 for general psychopathology and from 98.26±4.51 to 52.43±3.32 for total score[34].

Study No. 8 discussion and conclusion

In conclusion, the study reported that omega-3 fatty acids were effective on general psychopathology symptoms. Due to its favourable adverse effect, it would be a preferable add-on treatment and could potentially reduce the number of antipsychotics taken by patients[34].

6. DISCUSSION

6.1. Omega-3 and change in membrane phospholipids composition

Out of the nine studies reviewed, six studies measured RBC membrane phospholipids composition pre and post omega-3 fatty acid treatment. All six studies reported a significant increment of EPA after administration of EPA (Fig. 3). Two studies reported a significant increment of DHA after administration of DHA and Omega-3 fatty acids (containing both EPA and DHA), respectively. One study reported a significant increase in DHA after 2 g EPA administration; however, a significant decrease in DHA following administration of 1 g and 4 g of EPA, respectively. One study reported a significant increase in DHA only in low baseline PUFA patients following administration of EPA. One study reported a significant increase in DPA.

Decreased PUFA levels in membrane phospholipids have been associated with Schizophrenia. The above outcomes suggest that EPA supplement can increase membrane EPA and DPA but not DHA. The lack of DHA could probably be attributed to inadequate production of elongase and delta-6 desaturase enzymes, which could be explained by the build-up of DPA in van Rensburg et al., 2009 study[31]. Elongase and delta-6 desaturase are enzymes that take part in the conversion of EPA to the precursor DPA and then to DHA. Abnormality in these enzymes or lack of dietary PUFA intake are suggested to alter membrane phospholipid composition. It was suggested that dietary intake of PUFAs could modify the expression of genes associated with elongase and desaturase enzymes[31]. The lack of DHA could also be due to an abnormality in incorporation of DHA into membrane phospholipids in schizophrenic patients (which is supported by some other studies that reported schizophrenics' insensitivity to DHA supplements); or it could also be due to a competition between EPA and DHA for incorporation into phospholipids[20]. It was assumed that most patients with schizophrenia had a genetic abnormality in incorporation of essential fatty acids into membrane[31]. Similarly, several other omega-3 studies in healthy patients reported increased EPA and DHA in plasma following supplementation of fish oil that contained both EPA and DHA;

however, no increase in DHA was observed when supplementing pure EPA due to insufficient conversion of EPA to DHA in plasma[20].

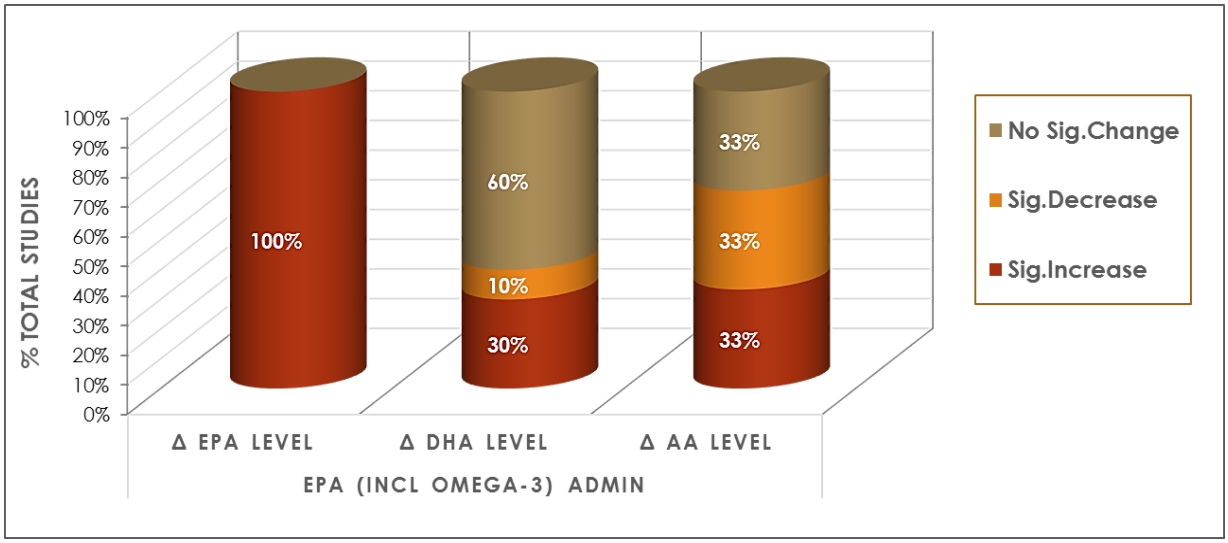

***Figure 3**. The figure shows change in membrane fatty acids post EPA (incl. omega-3 fatty acids) administration. Δ EPA & Δ AA represent 6 studies each. Δ DHA represents 5 studies.*

Six studies measured AA (including omega-3 measurements) and two of them found significant change in AA after omega-3 administration. Peet et al., 2002[29] reported that AA increased significantly after 2 g EPA administration but decreased significantly after 4 g EPA administration. Bentsen et al., 2013[33] reported a significant increase in AA after administration of sole EPA to patients with low baseline PUFA levels. Additionally, Fenton et al., 2001[28] and Amminger et al., 2010[32] reported a significant decrease in AA/EPA ratio and omega-6/omega-3 ratio following administration of 3 g EPA and 1.2 g omega-3, respectively; however, this could be due to increased EPA and thus the change in AA cannot be ruled out. PLA_2 enzymes release AA from membrane phospholipids for synthesis of eicosanoids. An increased PLA_2 activity and decreased AA level have been associated with schizophrenia. It was also suggested that the activity of PLA_2 could be supressed by EPA, which could lead to increased AA[31]. Moreover, there is a dose-dependent competition between EPA, DHA and AA for phospholipid incorporation, which could explain the increase in AA during supplementation of 2 g EPA but not during 4 g EPA[20].

Van Rensburg et al., 2009[31] measured other fatty acids, and reported a significant decrease in monounsaturated fatty acids and saturated fatty acids following treatment with 3 g EPA. The highest decrease was by 49.32% in the monounsaturated fatty acid, eicosaenoic acid. This suggests a possible

displacement of monounsaturated fatty acids and saturated fatty acids with EPA[31]. The abnormal proportion of saturated fatty acids to unsaturated fatty acid in membrane phospholipids has been linked to schizophrenia[27].

6.2. Omega-3 and symptom outcome

Seven out of nine studies reported improved symptoms following administration of omega-3 fatty acids (Fig. 4). Out of the seven studies, four reported significant improvement in positive symptoms and general psychopathology symptoms. Peet et al., 2001[27] (India) reported that eight patients out of fourteen on EPA had greater than 50% improvement in positive symptoms. Two studies reported significant improvement in negative symptoms. Six studies reported significant improvement in total scores. Additionally, in Peet et al., 2001[27] (UK) study, which is the only study that tested both EPA and DHA supplements, a significantly higher number of patients on EPA showed greater than 25% PANSS score improvement compared to DHA group. Overall, these outcomes suggest that omega-3 fatty acids might be slightly more beneficial to positive and general psychopathology symptoms than negative symptoms. It also indicates that EPA supplement has more benefits in improving symptoms than DHA alone does. This result is consistent with other studies with omega-3 fatty acids containing EPA only or combination of EPA and DHA, which consistently reported improved symptoms in schizophrenia and bipolar disorder[29].

One study (Bentsen et al., 2013)[33] reported worsening of positive symptoms in patients who had low baseline PUFA level after administration of EPA alone. When EPA was administered together with vitamin E and C, the low baseline PUFA patients showed no change in symptoms. Similarly, no change in symptoms was observed in patients with high baseline PUFA. The outcome from this study indicates that there is an association between baseline PUFA level and symptom outcome. Adding EPA alone to the low baseline PUFA profile could probably exacerbate the low PUFA level by increasing the oxidative stress and could lead to worsening of symptoms. Oxidative stress is known to impair neural functions and dopamine modulation as well as blocks NMDA receptors, which induce psychotic symptoms. Schizophrenia has been associated with increased oxidative stress due to abnormal anti-oxidative protection, one of them being glutathione peroxidase deficiency. The study suggested that the abnormality in anti-oxidative protection could probably be limited to low baseline PUFA schizophrenics, which could explain the symptom exacerbation in low baseline PUFA group only[33]. Additionally, previous healthy controlled studies reported uneven distribution of membrane PUFA in schizophrenic patients in which both significantly low

and moderately low PUFA profiles were observed[27]. The absence of symptom change when administering EPA with vitamin E/C was attributed to the potential mutual redox activity between the supplements[33].

Fenton et al., 2001[28] found no correlation between omega-3 fatty acid supplement and symptom change[28].

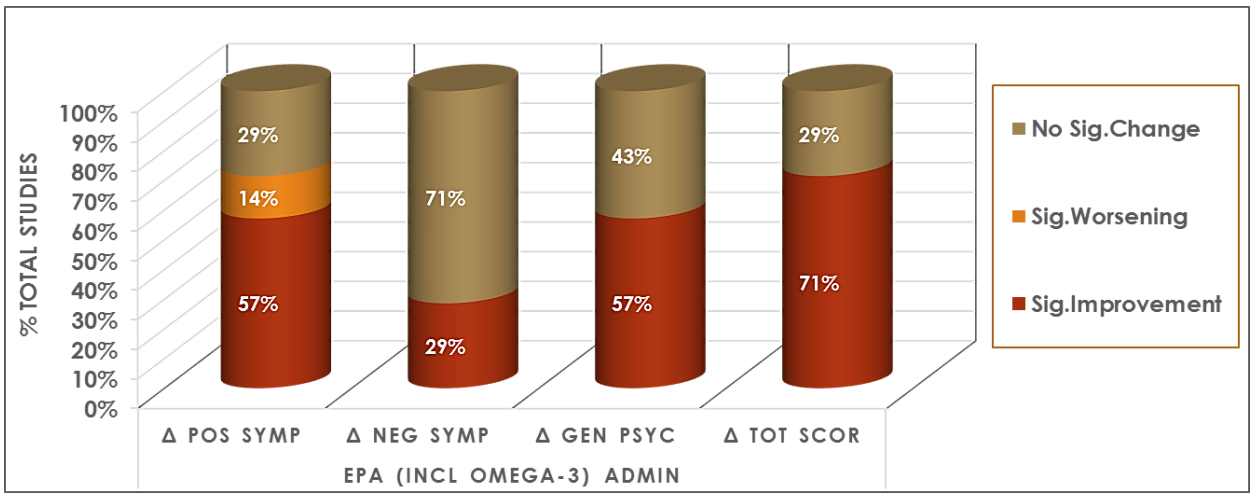

Figure 4. *The figure shows change in positive symptoms, negative symptoms, general psychopathology and total scores post EPA (including omega-3 fatty acids) administration. Each cylinder represents 8 studies.*

6.3. Correlation between membrane phospholipids composition change and symptom outcome

Six out of nine studies evaluated the association between membrane phospholipids profile and symptom outcome. Two out of six studies found correlation between increased EPA and symptom improvement (Fig. 5). Out of the two studies, one study (Peet et al., 2001, UK)[27] found correlation between increased EPA and positive symptoms improvement; one study (van Rensburg et al., 2009)[31] found correlation between increased EPA and improvement of negative symptoms and total PANSS score. No study reported a correlation between EPA profile change and general psychopathology symptoms. Additionally, none of the studies found association between DHA profile and symptom improvement. These outcomes suggest that symptom change is related to EPA profile rather than DHA. However, one out of five studies (Peet et al., 2002)[29] found no correlation between symptom change and EPA or DHA. Instead, they found correlation between total PANSS score improvement and increased AA following 2 g EPA supplement. The same study also reported less symptom improvement associated with decreased AA following 4 g EPA supplement[29]. In contrast, Bentsen et al., 2013[33] reported a correlation between worsened positive

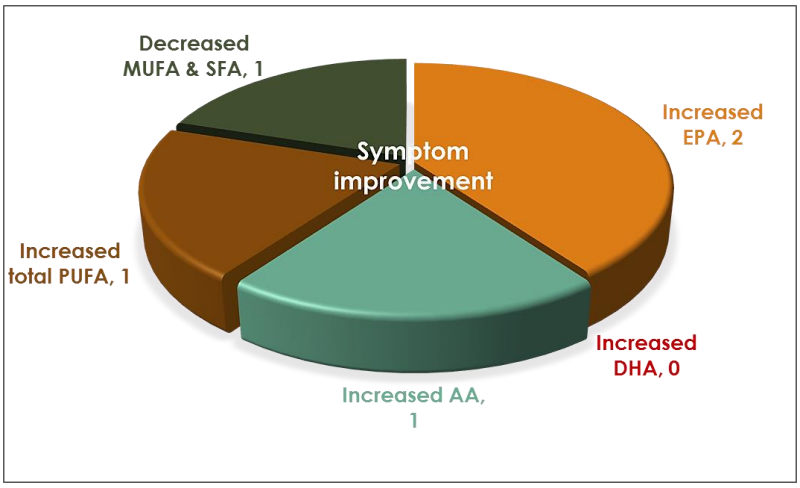

Figure 5. *The figure shows correlation between symptom improvement and membrane fatty acids change, and no. of studies that reported improved symptoms at least in one PANSS sub-scale*

symptoms and increased AA. Furthermore, although van Rensburg et al., 2009[31] did not observe a significant increase in AA, the patients that showed greater than 20% symptom improvement in EPA group had relatively higher AA profile than other patients did in the same group following EPA treatment[31].

The composition of membrane fatty acids have a significant impact on the overall function of cells. Membrane fatty acids regulate the fluidity of membranes and thereby affects the functions of membrane proteins[20] and receptors for neurotransmitters such as dopamine[27]. Gene expressions can also be affected by these fatty acids as they serve as ligands to nuclear receptors. However, membranes, mostly in neural tissues, are rich in DHA while EPAs are found in small amount. Actually, DHA is several hundred times more abundant than EPA in membranes of neural tissues[20]. Therefore, the symptom improvement shown in correlation with EPA but not DHA is probably not due to the direct modification of EPA in membrane phospholipids. It could be mediated by other factors such as AA. Even if EPA is found in small amount in neuronal membranes, it has several biological functions. For example, it takes part in synthesis of eicosanoids, influences gene expression, increases intracellular calcium level and inhibits PLA_2 enzymes. PLA_2 enzymes release AA from membrane phospholipids for synthesis of eicosanoids[27] and other pro-inflammatory mediators. In the absence of proper regulation of PLA_2 activity, abnormality in phospholipid metabolism occurs, which can induce oxidative stress. Schizophrenia has been linked to hyperactivity of PLA_2 enzymes and oxidative stress[31]. Khan et al., 2002[4] found a significant decrease in EPUFAs (especially AA and DHA) and an increased level of lipid peroxides (thiobarbituric acid reactive substances (TBARS)), in schizophrenics which suggested an increased lipid peroxidation due to oxidative stress[4]. Moreover, a variety of antipsychotic drugs has been shown to inhibit PLA_2 enzyme[27].

Van Rensburg et al., 2009[31] found correlation between improved total PANSS score and decreased monounsaturated fatty acids as well as decreased saturated fatty acids. The same study reported that patients who reached a desirable PUFA/saturated fatty acids ratio showed the highest symptom improvements[31]. It is known that membrane fluidity is affected by the saturation of membrane fatty acids. The highly polyunsaturated fatty acids, omega-3 and omega-6, can decrease saturated fatty acids and other lipids such as cholesterol[22][23], which increase membrane fluidity and thus promoting normal cell function[22][23][24].

Additionally, two studies found correlation between baseline membrane EPA level and symptom outcomes. Peet et al., 2001[27] reported that patients with greatest positive symptoms improvement had the highest baseline EPA level and those with low EPA responded less to treatment[27]. Similarly, Bentsen et al., 2013[33] reported that those with low baseline EPA had worsened positive symptoms, whilst those with high baseline EPA did not have symptom change following sole EPA treatment[33]. Patients with low baseline PUFAs are anticipated to have a more serious membrane phospholipid metabolic disorder, which may require more intervention than an omega-3 treatment[27]. It is also important to note that Khan et al., 2002[4] found a correlation between the lowest level of EPUFAs and the highest degree of illness in schizophrenics compared to healthy controls[4].

Two out of five studies (Fenton et al., 2001[28] and Amminger et al., 2010[32]) found no association between membrane phospholipids change and symptom improvement.

6.4. The effect of different omega-3 fatty acid dosages in membrane phospholipids change and symptom outcome

All studies, except for two, administered EPA alone as an omega-3 fatty acid treatment. One study (Amminger et al., 2010)[32] administered a combination of EPA and DHA. One study (Jamilian et al., 2014)[34] used 1 g omega-3 with unspecified dosage of EPA and/or DHA. In general, various dosages of EPA ranging from 700 mg to 4 g were used. All EPA dosages gave a significant increase in EPA profiles of membrane phospholipids. However, change in DHA profiles was inconsistent. 1 g and 4 g EPA gave a significant decline in DHA. 2 g EPA gave both significant and insignificant increase in DHA in two different studies. 700 mg and 3 g EPA gave insignificant increase in DHA. Similarly, 1 g EPA gave no change in AA profile. 2 g EPA gave significant increase in AA in one study, whilst it gave insignificant decline in another study. 3 g EPA gave a significant decline in AA in one study, whilst it gave insignificant increase in another study.

In relation to symptoms, all dosages resulted in improved symptoms (as applicable) except for 3 g EPA, where one study reported improved symptoms but another study reported no change in PANSS score.

In general, out of the three unsaturated fatty acids (EPA, DHA and AA), AA profile appears to show the most constant sensitivity to EPA dosage variance as its significant rise was only observed in 2 g EPA administration, whereas it declined or did not change significantly in other dosages. Peet et al., 2002[29] noted that the most consistent result was obtained in 2 g EPA and found a correlation between increased AA and symptom improvement in this dosage group. They also noted that the lack of symptom effectiveness in 4 g EPA group was due to reduction of AA in this dosage. EPA in 2 g could probably inhibit the overactive PLA_2 activity seen in schizophrenia, which in return increase the AA. EPA, DHA and AA are known to compete with each other for incorporation into membrane phospholipids[29], which could be the reason for reduction in AA in 3 g and 4 g EPA administration. However, it is important to note that Peet et al., 2001[27] UK reported an insignificant decrease in AA after administration of 2 g EPA[27]. Likewise, even though it is not as consistent as AA, the dose-dependent EPA, DHA and AA competition might also explain the similar sensitivity to EPA dosage that was observed in DHA profile. DHA decreased significantly in 1 g EPA and 4 g EPA administration, whereas as it increased significantly in 2 g EPA administration.

Peet et al., 2001[27], UK used unspecified gram of DHA for a sub-group of patients as an omega-3 fatty acid treatment[27].

6.5. The influence of duration of illness in the efficacy of omega-3 fatty acids

Six out of nine studies specified duration of illness. Average duration of illness ranged from 2 years to 23.1 years. There was no correlation found between duration of illness and change in membrane phospholipid or symptoms. However, it was noted that the average duration of illness for the active EPA group in Bentsen et al., 2013[33] study was 2 years, which is the shortest illness duration of all. This was the only group (and study) that experienced worsening of symptom in low baseline PUFA patients following EPA alone treatment. Amminger et al., 2010[32] had ultra-high risk subjects who were not yet transitioned to schizophrenia and observed change in membrane phospholipid and symptom improvement[32].

6.6. Omega-3 fatty acids as add-on vs. monotherapy and influence of concomitant antipsychotics

Only one study (Peet et al., 2001[27] India) used omega-3 fatty acids as monotherapy. All the remaining studies used omega-3 fatty acids as add-on treatment to current antipsychotic medications that patients

were taking. Although there was not as much data available for the monotherapy treatment as add-on, based on the current data available, no major difference in outcome was observed between the two types of treatment. In addition to this, Peet et al., 2002[29] analysed their study's outcomes in relation to patients' antipsychotic drugs and observed that patients on Clozapine had the highest symptom improvement from omega-3 fatty acid treatment compared to those on typical and other atypical antipsychotics. Contrary to this, Emsley et al., 2002[30] found that patients who received omega-3 fatty acids as add-on to typical antipsychotics had a better symptoms outcome than those on clozapine.

6.7. General discussion about compliance and its influence

Accurate method to verify that patients are taking study drug and are following study procedures as instructed are key to the success of these types of studies. In such patient population, there is a high probability of being negligence in taking study drug as per instructions. Some of the studies reported that compliance was checked through counting of pills; while others used PUFA measurement to monitor compliance. A few studies did not specify compliance method at all. Counting pills seems to be a less reliable method in this case. Additionally, omega-3 is a supplement that can be purchased from any drug store and is found in diets. Provided the majority of the patients were outpatient, patients' dietary or supplementary intake of PUFA should have been strictly monitored to avoid placebo effect. A few studies (e.g. Peet et al, 2002[29] and van Rensburg et al., 2009[31]) reported a placebo effect and/or no change between active and placebo groups and this was probably due to placebo groups taking PUFA supplement or increasing their dietary PUFA intake.

7. CONCLUSION

Omega-3 fatty acid supplement, specifically EPA, appear to increase membrane phospholipids' EPA level but not DHA in patients with schizophrenia. It also seems to improve PANSS score in some patients but the result is inconsistent. The correlation between membrane phospholipid composition change and symptom outcome is also inconsistent. Some studies found no correlation, whilst other studies found correlation with either EPA or AA profile. Furthermore, the mechanism for therapeutic effect of omega-3 fatty acids is still unknown. The potential role of PLA_2, AA and oxidative stress in the aetiology of schizophrenia and mechanism of action of omega-3 fatty acids is an interesting theory and require further investigation.

In conclusion, omega-3 fatty acids can be a safe and affordable supplement for treatment of schizophrenia. However, the studies reviewed in this project work are relatively small and the data is inconclusive. Therefore, additional studies with larger number of patients to confirm omega-3's efficacy as add-on and as a sole treatment as well as its effect on schizophrenics with low vs. moderate/high baseline PUFA levels would be valuable.

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APPENDIX A – Positive and Negative Syndrome Scale (PANSS)[35]

POSITIVE AND NEGATIVE SYNDROME SCALE (PANSS) RATING CRITERIA

GENERAL RATING INSTRUCTIONS

Data gathered from this assessment procedure are applied to the PANSS ratings. Each of the 30 items is accompanied by a specific definition as well as detailed anchoring criteria for all seven rating points. These seven points represent increasing levels of psychopathology, as follows:

1- absent
2- minimal
3- mild
4- moderate
5- moderate severe
6- severe
7- extreme

In assigning ratings, one first considers whether an item is at all present, as judging by its definition. If the item is absent, it is scored 1, whereas if it is present one must determine its severity by reference to the particular criteria from the anchoring points. The highest applicable rating point is always assigned, even if the patient meets criteria for lower points as well. In judging the level of severity, the rater must utilise a holistic perspective in deciding which anchoring point best characterises the patient's functioning and rate accordingly, whether or not all elements of the description are observed.

The rating points of 2 to 7 correspond to incremental levels of symptom severity:

- A rating of 2 (minimal) denotes questionable or subtle or suspected pathology, or it also may allude to the extreme end of the normal range.
- A rating of 3 (mild) is indicative of a symptom whose presence is clearly established but not pronounced and interferes little in day-to-day functioning.
- A rating of 4 (moderate) characterises a symptom which, though representing a serious problem, either occurs only occasionally or intrudes on daily life only to a moderate extent.
- A rating of 5 (moderate severe) indicates marked manifestations that distinctly impact on one's functioning but are not all-consuming and usually can be contained at will.
- A rating of 6 (severe) represents gross pathology that is present very frequently, proves highly disruptive to one's life, and often calls for direct supervision.
- A rating of 7 (extreme) refers to the most serious level of psychopathology, whereby the manifestations drastically interfere in most or all major life functions, typically necessitating close supervision and assistance in many areas.

Each item is rated in consultation with the definitions and criteria provided in this manual. The ratings are rendered on the PANSS rating form overleaf by encircling the appropriate number following each dimension.

PANSS RATING FORM

		absent	minimal	mild	moderate	moderate severe	severe	extreme
P1	Delusions	1	2	3	4	5	6	7
P2	Conceptual disorganisation	1	2	3	4	5	6	7
P3	Hallucinatory behaviour	1	2	3	4	5	6	7
P4	Excitement	1	2	3	4	5	6	7
P5	Grandiosity	1	2	3	4	5	6	7
P6	Suspiciousness/persecution	1	2	3	4	5	6	7
P7	Hostility	1	2	3	4	5	6	7
N1	Blunted affect	1	2	3	4	5	6	7
N2	Emotional withdrawal	1	2	3	4	5	6	7
N3	Poor rapport	1	2	3	4	5	6	7
N4	Passive/apathetic social withdrawal	1	2	3	4	5	6	7
N5	Difficulty in abstract thinking	1	2	3	4	5	6	7
N6	Lack of spontaneity & flow of conversation	1	2	3	4	5	6	7
N7	Stereotyped thinking	1	2	3	4	5	6	7
G1	Somatic concern	1	2	3	4	5	6	7
G2	Anxiety	1	2	3	4	5	6	7
G3	Guilt feelings	1	2	3	4	5	6	7
G4	Tension	1	2	3	4	5	6	7
G5	Mannerisms & posturing	1	2	3	4	5	6	7
G6	Depression	1	2	3	4	5	6	7
G7	Motor retardation	1	2	3	4	5	6	7
G8	Uncooperativeness	1	2	3	4	5	6	7
G9	Unusual thought content	1	2	3	4	5	6	7
G10	Disorientation	1	2	3	4	5	6	7
G11	Poor attention	1	2	3	4	5	6	7
G12	Lack of judgement & insight	1	2	3	4	5	6	7
G13	Disturbance of volition	1	2	3	4	5	6	7
G14	Poor impulse control	1	2	3	4	5	6	7
G15	Preoccupation	1	2	3	4	5	6	7
G16	Active social avoidance	1	2	3	4	5	6	7

SCORING INSTRUCTIONS

Of the 30 items included in the PANSS, 7 constitute a **Positive Scale**, 7 a **Negative Scale**, and the remaining 16 a **General Psychopathology Scale**. The scores for these scales are arrived at by summation of ratings across component items. Therefore, the potential ranges are 7 to 49 for the Positive and Negative Scales, and 16 to 112 for the General Psychopathology Scale. In addition to these measures, a Composite Scale is scored by subtracting the negative score from the positive score. This yields a bipolar index that ranges from –42 to +42, which is essentially a difference score reflecting the degree of predominance of one syndrome in relation to the other.