**A randomised double-blind placebo-controlled trial of minocycline and/or omega-3 fatty acids added to treatment as usual for At Risk Mental States: The NAYAB Study.**

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**Abstract**

Background: Inflammatory mechanisms are thought to contribute to the onset of psychosis in persons with an at-risk mental state (ARMS). We investigated whether the anti-inflammatory properties of minocycline and omega-3 polyunsaturated fatty acids (omega-3), alone or synergistically, would prevent transition to psychosis in ARMS in a randomised, double-blind, placebo-controlled trial.

Methods: 10,173 help-seeking individuals aged 16-35 years were screened using the Prodromal Questionaire-16. Individuals scoring >5 were interviewed using the Comprehensive Assessment of At-Risk Mental States (CAARMS) to confirm ARMS. Participants (n=326) were randomised to minocycline (n= 82), omega-3 (n = 80), combined minocycline and omega-3 (n = 82) or to double placebo (n = 82) for a period of 6 months. The primary outcome was transition to psychosis at 12 months.

Findings: Forty-five (13·8%) participants transitioned to psychosis. The risk of transition was non-significantly greater in those randomised to omega-3 alone or in combination with minocycline (16.7%), compared to 10.4% in those not exposed to omega-3 (OR=1·81; 95% CIs 0.95, 3·45; p = 0·07). The OR for transitions on minocycline vs. no minocycline was 0·84 (95% CIs 0·45, 1·57; p>0.1). In participants who did not become psychotic, CAARMS and depression symptom scores were reduced at six and twelve months (mean CAARMS difference = 1·43; 95% CI: 0·33, 1·76; p = 0·004) in those exposed to omega-3. There were no effects of minocycline on CAARMS or depression scores.

Interpretation: Neither minocycline nor omega-3, either alone or combined, reduced transition to psychosis in ARMS. There was some evidence that omega-3 supplementation may benefit non-psychotic general and transdiagnostic symptoms.

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**Research in context**

Evidence before this study

Inflammatory processes have been implicated in the development of ARMS and schizophrenia. The antibiotic minocycline and dietary supplement omega-3 polyunsaturated fatty acids have anti-inflammatory and possible neuroprotective properties. We searched PubMed using the terms (omega-3 OR minocycline) AND (schizophrenia OR psychosis) to find randomised placebo-controlled trials in ARMS and in those with clinical disorder before 31st March 2017.

We found five trials of minocycline but none in ARMS. Four of the five trials targeted negative symptoms and one targeted persistent psychotic symptoms in patients on clozapine. All reported small but statistically significant benefits. Our study in early psychosis was the largest and randomised 90 patients in Pakistan and Brazil with an overall benefit on negative symptoms. Minocycline was equivalent to placebo for all-cause discontinuation and for discontinuation because of adverse events.

We found two omega-3 trials to prevent psychosis onset in ARMS with mixed results. The first, ‘VHR’ study, trialled a twelve-week intervention of omega-3 or placebo followed by 40-weeks monitoring. At 12 months, 2 of 41 individuals (4·9%) in the omega-3 group and 11 of 40 (27·5%) in the placebo group had transitioned to a psychotic disorder (p = 0·007).   In a subsequent study, ‘NEURAPRO’ (n=304), which investigated whether omega-3 in combination with cognitive behavioural case management (CBCM) the was more effective than placebo plus CBCM, the researchers did not detect a difference in transition to psychosis at six months. However, the lower-than-expected transition rate (10·5% at 12 months) may have prevented a test of the main hypothesis. There had been no studies from low and middle-income countries (LMIC) in ARMS using omega-3 or minocycline. There have been no clinical trials of the possible synergy of combining anti-inflammatory treatments such as omega-3 and minocycline in ARMS or in established psychotic disorders.

Added value of this study

The NAYAB study is the largest double-blind, placebo-controlled trial of omega-3 in an ARMS population, the first to test minocycline and the first in a LMIC. Furthermore, the factorial design evaluated, uniquely, whether two anti-inflammatory drugs might usefully synergise to reduce psychosis onset. The results clearly indicate that neither minocycline nor omega-3, either alone or in combination, reduced transition to psychosis at twelve months in an ARMS population. In individuals who did not become psychotic, omega-3 was associated with a statistically significant but small reduction in ARMS and depressive symptoms.

Implications of all the evidence

Neither minocycline nor omega-3 should be further investigated in an ARMS population without biomarker evidence of a potentially responsive subgroup.

**Introduction**

Schizophrenia is typically preceded by a prodromal phase and clinical criteria have been developed to identify individuals at high risk of developing a psychotic disorder such as the at-risk mental state (ARMS) (1) or clinical high-risk (2). Initial studies suggested that approximately 35% of individuals with ARMS develop a psychotic illness within a year (3) but these rates have fallen, with a recent meta-analysis suggesting that this figure is closer to 15% (4). Although most individuals with ARMS will not transition to psychosis, many will experience depression and persistent ARMS symptoms (5). Consequently, the ARMS period provides an opportunity to intervene to prevent or delay conversion to psychosis and improve longer-term outcomes.

Several preventative treatments have been evaluated in clinical trials for the ARMS population. Cognitive behaviour therapy has the strongest evidence base for efficacy in preventing transition to psychosis compared to treatment as usual but not in comparison with other psychosocial therapies, antipsychotic or experimental drug treatments (6,7). However, there are too few trials to draw definitive conclusions about overall or comparative efficacy of specific treatments. Three RCTs have evaluated dietary supplementation with omega-3 polyunsaturated fatty acids (omega-3) in the prevention of the onset of psychosis (8–10). The first placebo-controlled trial of preventative omega-3 supplementation in ARMS, VHR study (8), reported a transition rate of 11 of 40 (27·5%) in the placebo group compared to 2 of 41 (4·9 %) in the omega-3 group at twelve month follow up (p = 0·007). The NAPLS study (9) and the NEURAPRO study (10), failed to show significant effects of omega-3 supplementation on transition rate although the NAPLS study found a significant association between a baseline diet low in omega-3 foods and later conversion to psychosis.

The rationale for the use of omega-3 was to reverse supposed dietary deficiencies in psychosis that affect the lipid composition and properties of cell membranes and more recently that they inhibit cyclo-oxygenase 2 and formation of inflammatory prostaglandins (11). Studies of circulating cytokines indicate schizophrenia is associated with mild activation of the peripheral immune system (12) which might be a cause or consequence of inflammation in the brain. The possibility that an inexpensive and safe dietary supplement might reverse inflammatory-metabolic mechanisms of psychosis was of particular interest to our evaluation of treatments in Pakistan; a LMIC with prevalent poverty, communicable disease and food insecurity predisposing to dietary deficiency and inflammatory disorders.

We included a minocycline treatment arm as early studies in schizophrenia reported benefit on negative symptoms in persons with established and recent-onset schizophrenia (13) including a study from our group in Pakistan with a centre in Brazil (14). The selection of minocycline was encouraged by its specific ability to reduce inflammatory activation of microglia – the brain’s resident inflammatory cells (15). In addition, there were early reports of microglial activation in patients with schizophrenia, detected in-vivo using positron emission tomography (PET), to image radioligand binding to activated microglia (16). During the course of the NAYAB study there were two large scale studies of minocycline in schizophrenia that found no effect of minocycline on negative symptoms of schizophrenia (17) or overall symptoms of schizophrenia (18). However, these studies do not exclude the possibility that minocycline may be effective during a prodromal phase when inflammatory mechanisms may be most active in the pathogenesis of psychosis and there had been no studies of minocycline in an ARMS population to assess for prevention or delay to psychosis transition.

We carried out a double-blind randomised placebo-controlled trial of minocycline and omega-3 to determine their efficacy in preventing transition to psychosis in persons with ARMS. Omega-3 and minocycline are safe and widely available drugs in LMICs. Both drugs have anti-inflammatory actions and we hypothesised they might, either individually or synergistically, be effective in preventing transition to psychosis by reducing neuroimmune mechanism of risk.

**Methods**

Study design

NAYAB was a multicentre, double-blind, randomised placebo-controlled study of minocycline and omega-3 using a 2 x 2 factorial design. The four treatment groups took either minocycline, omega-3, their combination, or their matching placebos for 6 months followed by a 6-month follow-up period. The study was conducted within three large cities in Pakistan (Lahore, Karachi, and Rawalpindi) with a combined population of approximately forty-six million. The trial was conducted between March 2017 and November 2018. The hypotheses and full study protocol have been published (19). Ethical approval for the study was obtained prior to study commencement from the Ethics and Scientific Review Committee of the Karachi Medical and Dental College, Pakistan.

Participants and selection of primary outcome measure

Eligible participants were aged 16–35 years who met criteria for one or more of the three forms of ARMS: state vulnerability, attenuated psychotic symptoms or brief limited intermittent psychotic symptoms. Symptoms and criteria were assessed using the Comprehensive Assessment of At Risk Mental States (CAARMS) (20). The CAARMS is a widely used and validated semi-structured interview for identification of individuals at increased risk of developing a first-episode psychotic disorder. Ratings of the severity of four groups of positive psychotic symptoms can be summed to produce a continuous ‘global’ severity measure or added to the sum of the four frequency ratings to produce a ‘combined’ measure. The distress associated with severity ratings is assessed by a visual analogue rating.

Participants were excluded if there was a history of psychotic illness; diagnosis of learning disability; any pre-existing inflammatory conditions (e.g. rheumatoid arthritis); organic brain disease (e.g. epilepsy); previous treatment with an antipsychotic, mood stabilizing agent or electroconvulsive therapy; prior history of intolerance or serious side effects to tetracycline or omega-3; concomitant penicillin therapy or anticoagulant therapy (to minimise adverse drug events); active substance abuse (except nicotine or caffeine) or dependence within the last three months according to Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (21); pregnant or breastfeeding; current or previous treatment with either a tetracycline or omega-3 in the three months before study entry; prescribed anti-inflammatory medication at study entry; active expression of suicidal ideation or current aggressive/dangerous behaviour; relevant current or past hematologic, hepatic, renal, neurologic or other medical disorder that might interfere with the study. All participants gave written informed consent to participate.

The study was widely advertised to the general population using radio and newspaper articles, general practitioner engagement and self-referrals from potential participants and family members. There are no early intervention or other youth mental health services in Pakistan. We screened for the presence of prodromal symptoms using the Prodromal Questionnaire-16 (PQ-16), a brief self-report questionnaire. We used a cut-off value of 6 that has been shown to have high sensitivity (87%) and specificity (87%) in identifying ARMS (22). Participants with scores of 6 or above were subsequently assessed with the CAARMS to identify those meeting criteria for ARMS. Screening and recruitment to the study was undertaken by a team of trained researchers.

Randomisation and masking

Participants were randomly assigned in a 1:1:1:1 ratio to either placebo, minocycline, omega-3 or minocycline and omega-3 in combination. The trial pharmacist prepared study medicines according to the randomisation code generated by the study statistician in the UK (SL). Research assistants (RAs) delivered study medication to participants. Participants received an equivalent number of over-encapsulated tablets and placebo capsules were manufactured to match the active study drugs. Pill counts were undertaken by RAs to assess adherence and no participant received less than 75% of the allocated intervention. Participants, research assistants undertaking the assessment measures and the trial statistician were blind to treatment allocation.

Procedures

During the first week of the study participants received a 100 mg capsule of minocycline daily (or matching placebo) then 200 mg of minocycline daily (or matching placebo) for the remaining intervention period; all participants tolerated the 200 mg. We chose to trial 200 mg as this was the dose used in a previous trial in early-phase schizophrenia (17). Two 600 mg capsules of omega-3 gave a daily dose of 1·2 g (720 mg of eicosapentaenoic acid, EPA, and 480 mg of docosahexaenoic acid, DHA) based on trials in an ARMS population (10). Omega-3 has a soft-shell capsule and minocycline is a hard-shell capsule with different appearances, so hard and soft-shell matching placebos were provided such that all participants received 2 hard and 2 soft capsules after titration. Research assistants obtained informed consent to the procedures described in the patient information leaflet. No participant received concurrent psychotropic medication or psychological treatment during the study. Participants were seen on four occasions: at baseline, 3, 6, and 12 months after study entry for research assessments measures.

Outcomes

The primary efficacy outcome was conversion to psychotic disorder at 12 months after study entry. This was operationally defined using the positive symptom subscales in the CAARMS as one of:-

1. Unusual thought content held with delusional intensity (global score 6) occurring several times or more per week (frequency and duration score > 3).
2. Non-bizarre ideas held with delusional conviction (global score 6) occurring several times or more per week (frequency and duration score > 3).
3. Perceptual abnormalities in any modality (global score ≥ 5) occurring several times or more per week (frequency and duration score > 3).
4. Disorganised speech (global score ≥ 5) occurring several times or more per week (frequency and duration score > 4).

Secondary outcome measures included total CAARMS global positive symptom scores post-treatment at 6 and 12 months co-varying for baseline; change in social and occupational functioning at 12 months using Social and Occupational Functioning Assessment Scale (SOFAS) (23); change in severity of depressive symptoms between study entry and at 12 months measured by the Montgomery-Åsberg Depression Rating Scale (MADRS) (24). Adverse events and serious adverse events were monitored throughout the study and assessed at each visit during the intervention phase.

Statistical Analysis

The study was powered based on an expected transition rate to psychosis of 30% in the placebo group. We expected a higher transition to psychosis rate in an ARMS population in Pakistan because of the risk exposures mentioned and the poor access to psychosocial interventions and pharmacotherapy. For the study interventions to be considered clinically beneficial, a reduction in transition to psychosis to 15% was deemed necessary. This required a sample size of 59 per treatment arm to detect placebo-active differences with a power of 80% and at the 5% significance level. To allow for a 25% drop-out rate and to ensure there were 59 completing participants per arm, a minimum sample size of 80 subjects per treatment group was required requiring a total sample size of 320 participants.

The statistical analysis was overseen by study statistician (SL). There were no interim analyses and all analyses were carried out after the collection of the final outcome measures. All statistical analyses of the clinical outcomes were performed using STATA v14 and SPSS v27. When the primary analyses were complete, the treatment code was revealed. Treatment effects on the primary outcome and other categorical outcomes were calculated as odds ratios (OR) and 95% confidence intervals with single chi-square tests of statistical significance across the 4 treatment groups.

The factorial analysis for omega-3 compared psychosis onsets in ‘all omega-3’ exposed participants whether taken alone or in combination with minocycline, versus the ‘no-omega-3’ participant group taking double placebo or minocycline alone. Similarly, psychosis onsets in the ‘all minocycline’ exposed participant group, defined as minocycline alone or in combination with omega-3, were compared with ‘no minocycline’ groups taking double placebo or omega-3 alone. We used analysis of variance to analyse the post-treatment ratings at 6 and 12 months (repeated ‘time’ measure) co-varying for baseline with fixed factors for omega-3 (‘all omega-3’ vs. ‘no omega-3’) and minocycline (‘all minocycline’ vs. ‘no minocycline’). We looked for evidence of synergy in positive interactions between omega-3 and minocycline factors. Last observations for drop-outs were carried forward to the post treatment ratings.

This study was registered in October 2015 (ClinicalTrials.gov identifier: NCT02569307)

Role of the funding source

The study was funded by the Stanley Research Medical Institute. The funder had no role in study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

**Results**

Between March 2016 and November 2017, 326 participants were recruited and randomised to the study from a screened sample of 10699. The last 12-month visit was in November 2018. Figure 1 shows participant flow in the trial. 80-82 participants were randomised to each of the four treatment groups. 29 participants dropped out of the study resulting in a 91% retention rate. Drop-outs were evenly distributed between the treatment groups with the exception that 4 participants in the omega-3 + placebo group self-harmed by overdose or self-cutting, requiring brief medical attention but not continued contact with health services. 27 of the 29 participants who dropped out did so before their scheduled first follow-up at 3 months; their baseline clinical ratings were carried through the follow-ups for the secondary, intention-to-treat analysis of symptoms and function ratings.

Figure 1 Trial profile



Note: Each time-point indicates when CAARMS assessments were carried out. “Psychosis” indicates those meeting the CAARMS threshold criteria.

The randomised sample included 60% male participants with a mean age of 24 years at trial entry. All participants rated positive for attenuated psychotic symptoms (APS) and none had brief limited psychotic symptoms (BLIPS). 15% met criteria for the vulnerability risk group, defined by family history and declining performance.

There were no statistically significant or numerically important differences in age, sex or other social and occupational demographics between the 4 treatment groups (Table 1) nor between the 29 dropouts and study completers (Supplementary table 1). The baseline mean ratings for psychosis (CAARMS), depression (MADRS), and functional impairment (SOFAS) point to mild-moderate severity across the sample.

Table 1:

Baseline demographics and outcome variables for 4 intervention groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Placebo+ Placebo (n=82)Mean (SD) | Omega 3 +Minocycline (n=82)Mean (SD) | Minocycline+ Placebo (n=82)Mean (SD) | Omega 3 +Placebo (n=80)Mean (SD) |
| Age | 23·9 (5·3) | 24·4 (5·3) | 25·2 (5·3) | 23·8 (5·4) |
| Education |  9·5 (4·5) | 9·5 (4·3) | 8·6 (5·4) | 8·5 (4·9) |
| PQ-16 (0-16) | 9·3 (1·5) | 9·1 (1·7) | 9·1 (1·6) | 8·9 (1·5) |
| CAARMS Psychosis |  |  |  |  |
|  Global (0-24) | 13·8 (1·8) | 13·9 (2·0) | 13·8 (2·1) | 14·1 (1·7) |
|  Combined (0-48) | 27·7 (3·6) | 27·8 (3·9) | 27·7 (3·7) | 27·6 (3·4) |
|  Distress (0-100) | 60.8 (1.5)  | 60.7 (1.9) | 60.3 (1.7) | 61.6 (1.5) |
| MADRS (0-60) | 27·6 (8·5) | 28·0 (8·6) | 26·7 (8·4) | 27·4 (9·2) |
| SOFAS (0-10) | 6·6 (0·6) | 6·7 (0·6) | 6·6 (0·6) | 6·6 (0·7) |
|  |  |  |  |  |
|  |  N (%) |  N (%) |  N (%) |  N (%) |
| Sex |  |  |  |  |
|  Male | 50 (61·0) | 47 (57·3) | 48 (58·5) | 47 (58·8) |
|  Female | 32 (39·0) | 35 (42·7) | 34 (41·5) | 33 (41·3) |
| Marital Status |  |  |  |  |
|  Single | 56 (68·3) | 55 (67·0) | 49 (59·8) | 56 (70.0) |
|  Married | 24 (29·3) | 27 (32·9) | 31 (37·8) | 23 (28·8) |
|  Divorced  | 2 (2·4) |  0  | 2 (2·4) | 1 (1·3) |
| Family System |  |  |  |  |
|  Nuclear | 38 (46·3) | 42 (51·2) | 36 (43·9) | 41 (51·3) |
|  Extended  |  44 (53·7) |  40 (48·8) |  46 (56.0) |  39 (48·8) |
| Employment |  |  |  |  |
|  Unemployed | 28 (34·2) | 26 (31·8) | 29 (35·4) | 27 (33·8) |
|  Employed | 29 (35·4) | 33 (40·2) | 37 (45·1) | 32 (40.0) |
|  Student  |  25 (30·5) |  23 (28·0) |  16 (19·5) |  21 (26·3) |

Key: PQ-16 = Prodromal Questionnaire; CAARMS= Comprehensive Assessment of At-Risk Mental States; MADRS= Montgomery–Åsberg Depression Rating Scale; SOFAS= Social and Occupational Functioning Assessment Scale

Forty-five (13·8%) participants transitioned to psychosis; 44 assessed via CAARMS criteria and one who was admitted with a psychotic illness prior to the first 3-month CAARMS interview (Figure 1). All but 7 of the transitions to psychosis had occurred by 3 months and none occurred after 6 months. The rate of psychosis onsets in the double placebo group was low at 11·0% and marginally lower at 9·8% in the minocycline alone group whereas onsets were greater in the omega-3 alone (17·1%) and the combined minocycline + omega-3 group (18·3%) (Table 2). These group differences in proportion were not statistically significant by chi-square.

**Table 2: Conversion to psychosis by intervention**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Placebo +Placebo (n=82)N (%) | Omega-3 + Minocycline (n=82)N (%) | Minocycline + Placebo (n=82)N (%) | Omega-3 +Placebo (n=80)N (%) |
| 3-month | 6 (7·3) | 12 (15·9) | 7 (8·5) | 13 (15·9) |
| 6-month | 3 (3·7) |  1 (1·2) | 1 (1·2) | 2 (2·4) |
| 12-month |  0  |  0 |  0 |  0 |
| Total | 9 (11·0) | 13 (17·1) | 8 (9·8) | 15 (18·3) |

**Table 3: Conversion to psychosis by total minocycline or omega-3 exposures; factorial analysis**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | No Minocycline(n=164)N (%) | All Minocycline(n= 162)N (%) |  | No Omega-3(n=164)N (%) | All Omega-3(n=162)N (%) |
| 3-month | 19 (11.6) | 19 (11·7) |  | 13 (7·9) | 25 (14·8) |
| 6-month | 5 (3.1) | 2 (1.2) |  | 4 (2·4) | 3 (1·9) |
| 12- month |  0 |  0  |  |  0  |  0  |
| Total | 24 (14·6) | 21 (12·3) |  | 17 (10·4) | 28 (16·7) |

Key:

No Minocycline group comprised Placebo + Placebo and Omega-3 + Placebo treatment groups

All Minocycline group comprised Minocycline + Placebo and Omega-3 + Minocycline treatment groups

No Omega-3 group comprised Placebo + Placebo and Minocycline + Placebo treatment groups

All Omega-3 group comprised Omega-3 + Placebo and Omega 3 + Minocycline treatment groups

In the factorial analysis (Table 3), the 28 onsets in the 162 ‘all omega-3’ participants (16.7%) was greater than the 17 in the ‘no omega-3’ group (10·4% of 164) but this was not a statistically significant aggravation of risk; OR=1·81 (95% CIs 0.95, 3·45; chi square p = 0·07). Of the 162 ‘all minocycline’ participants, 21 developed psychosis compared to 24 of the 164 in the ‘no minocycline’ group; an OR close to unity (OR = 0·84, 95% CIs 0·45, 1 ·57).

The 45 participants who developed psychosis showed closely similar baseline demographics and clinical ratings compared with those who did not become psychotic (supplementary table 1). However, only one third of the onset cases compared to half of the no-onset cases came from nuclear families (p = 0·03). There was no indication of impending psychosis in raised CAARMS ratings at 3 months of the 7 participants who were psychotic by 6 months.

Figure 2 shows the time course of the CAARMS total ratings in the four treatment groups, carrying forward the baseline ratings of 27 of the 29 dropouts and the last (3 month) observation of the remaining 2. There was a sharp fall from baseline CAARMS scores to 3 months and little further change to end of treatment ratings at 6 months and follow-up at 12 months. Repeated measures analysis of variance of the post-treatment CAARMS global positive symptom ratings (6 and 12 months), with baseline a covariant, revealed a small, apparently beneficial effect of omega-3 exposure on CAARMS scores. Participants who received omega-3, either alone or combined with minocycline; (the ‘all omega-3’ group) had significantly lower global CAARMS scores at 6 and 12 months compared to the ‘no omega-3’ group (mean difference = 1·43; 95% CIs: 0·33, 1·76; p =·004; fig 2). The omega-3 effect was equally statistically significant for the Combined and Distress CAARMS scores. There were no main effects or interactions with time (6 vs. 12 month) for minocycline (‘all minocycline’ group vs ‘no minocycline’ group).

Figure 2 Time course of CAARMS global positive symptom ratings



Key: Blue lines represent participants who received omega-3. Red lines those who did not receive omega-3.

6 and 12 month post-treatment scores include baseline scores of 29 dropouts carried forward. CAARMS standard errors all less than 0·5. Main effect of omega-3 on 6 &12 months CAARMS, p=·004.

The diminished mean CAARMS scores in the’ all omega-3’group, relative to the ‘no omega-3’ group, could reflect the greater loss to psychosis in the ‘all omega-3’ group by reducing the proportion of high CAARMS scorers. We therefore compared the groups after excluding all high CAARMS scores from both groups (CAARMS >10; see histograms in supplementary Fig 1). Low scores at outcome remained significantly over-represented in the ‘all omega-3’ group compared to the ‘no omega-3’ group (Chi-square p=0·01). Exposure to omega-3 was also associated with better MADRS depression ratings which were lower in the ‘all omega-3’ group than in the’ no omega-3’ group at the end of treatment at 6 months but not at 12 month follow-up (treatment by time interaction p=0·04).

We explored whether risk group status changed with treatment. There were no BLIPS at entry or in follow-up visits. All participants met APS criteria at baseline and 80% met APS criteria at 12 months. At 12 months, 15·8% of the ‘all omega-3’ group and slightly fewer (6·8%) in the ‘no omega-3’ group, had become APS free, a significant group difference against the 100% baseline of the randomised sample (chi-square p = 0·045). Social and occupational impairment (SOFAS ratings) showed little change in those who remained in the study over the 12 months; 13·1% improved by 2-3 categories and 14·6% were rated with good or slight impairment in contrast to none at baseline. SOFAS scores were unaffected by treatment group. There were no group differences in adverse events.

**Discussion**

The aim of the trial was to test the hypothesis that an inflammatory process mediates the onset of psychosis in those at high risk of developing psychosis. We tested the prediction that six months treatment with minocycline and/or omega-3, either alone or combined, would reduce the transition to psychosis over 12 months in comparison to placebo in an ARMS population defined by CAARMS criteria. We found no evidence that treatment with minocycline or omega-3, either alone or in combination, reduced transition to psychosis and nearly all participants at 12 months continued to fulfil criteria for ARMS.

Our findings are consistent with a large trial of minocycline in recent-onset schizophrenia that found no antipsychotic benefit of minocycline (17). Epidemiological studies have not found a protective effect of minocycline exposure in adolescence on later risk of psychosis (25) also in keeping with the results of the present study. In-vivo imaging of the microglial marker protein TSPO using PET increasingly suggests that microglial inflammation occurs in depression rather than in schizophrenia and recent studies suggest minocycline has antidepressant efficacy ﻿(26,27). In schizophrenia, reductions in TSPO expression are consistently reported which are most marked in studies with recent onset, drug-naïve patients (28) and in the absence of an inflamed microglial phenotype in the ARMS, a beneficial effect of the anti-microglial properties of minocycline seems improbable.

Omega-3 was not effective in reducing rates of transition to psychosis in the present study and this is in keeping with the large NEURAPRO study (11). In both the NEURAPRO and NAYAB studies, the low transition rates in placebo-treated participants (14% in NAYAB study and 11% in NEURAPRO) might have reduced power to detect a preventative effect. However, there is no indication of an effect of omega-3 over placebo that could be rendered statistically significant with the greater power of a larger sample. Furthermore, in the present sample, treatment with omega-3 alone or in combination with minocycline was associated with a greater number of transitions than those not receiving omega-3 at a trend level of statistical significance by the 12-month endpoint. This is probably a chance finding since there are no clear precedents in the literature bar one report in which EPA supplementation without the DHA component of omega-3, increased drop-outs during treatment for a psychotic episode (29). Psychotic symptoms did not increase except, counterintuitively, in a subgroup with low blood omega-3 PUFA concentrations. The age of the sample treated could be a determinant of the efficacy of omega-3. The mean age of the present sample was 24·4 years which contrasts with 16·8 years in the VHR study (8) in which omega-3 reduced transitions, and 19·4 years for the negative NEURAPRO study (10). If these ages reflect duration of a prodromal state, it might be that omega-3 promotes recovery in those with a short prodrome (younger age) but not those with a long prodrome. If instead younger ages reflect earlier age of onset of illness, it could suggest that omega-3 has a beneficial effect on the developing brain, regardless of duration of prodrome. However, in the absence of information of age of first symptom, it is not possible to speculate further

Despite the lack of benefit on transitions, those exposed to omega-3 showed a statistically significant improvement in CAARMS and MADRS ratings in those who did not reach the threshold for psychosis. An obvious explanation is that more participants with high scores might have been lost to the omega-3 groups through their greater rate of transitions. However, this is not the case because those who became psychotic did not have greater baseline CAARMS or MADRS scores (supplementary table 1) and, furthermore, omega-3 treatment was associated with an absolute increase in the numbers with low final CAARMS scores (supplementary figure 1). Two studies in early active psychosis reported omega-3 improved non-psychotic symptoms including depression (30,31) and general psychopathology and function (30). There is increasing evidence that low PUFA levels occur as much in major depression as in schizophrenia (32) and in ARMS a high omega-6 to omega-3 ratio predicts subsequent mood disorders and not psychosis (33). A meta- analysis of double-blind RCTs ( n= 2160) found therapeutic benefits of omega-3 in major depressive disorder (34) and on depressive and general symptoms rather than on positive and negative symptoms in first episode psychosis (29). These findings together with the small reductions in CAARMS and MADRS ratings in the non-psychotic participants in NAYAB suggest that omega-3 supplementation may benefit a transdiagnostic, non-psychotic general symptom domain without benefit on transition to psychosis in persons with an ARMS or FEP.

**Strengths**

The current study is the first to trial minocycline with omega-3 PUFAs at scale in an ARMS population. A strength of this study was the large sample size in comparison to previous pharmacological intervention studies in this area that have been in the range n = 44-304 (6) and the use of a validated assessment measure in terms of ascertainment of ARMS and transition to psychosis. Recruitment to the study was high in those assessed as CAARMS positive with almost all (98%) consenting to randomisation indicating trial acceptability and a willingness to take part, indicating viability of future trials in similar population in a LMIC. Attrition rates were less than 10% in all study arms making for minimal possible confounding by undetected missing at random assumptions and demonstrating the study interventions were highly acceptable.

**Limitations**

Longer duration of prodromal symptoms is a risk factor for transition to psychosis but was not measured. Only the positive sub-scale of CAARMS was measured which despite being of particular interest, does not capture the full range of clinical symptoms in an ARMS population. However, we supplemented the positive sub-scale of CAARMS with the MADRS. It is possible that the drugs could have a detectable benefit in reducing risk of transition if an ARMS group could be defined, possibly using biomarkers, with a substantially greater risk of transition.

**Conclusion**

We found no evidence that either minocycline or omega-3 PUFAs, either alone or in combination, reduce a putative inflammation-mediated transition to psychosis in an ARMS population. There was evidence that omega-3 supplementation had a small non-specific benefit on positive psychotic symptoms and mood ratings in those who did not become psychotic. Further omega-3 studies and minocycline studies are not indicated until it has been demonstrated that a substantial subgroup exists with reliably reduced erythrocyte omega-3 PUFA or with immune activation that have a high rate of transition.

**Contributors**

IBC, BD, NH, IQ conceived the idea for the study, obtained the funding and are responsible for the decision to submit for publication. AY advised on the trial design and use of the CAARMS. Data collection was overseen by ABK and primary data analysis was undertaken by SL. DH and BD carried out supplementary analyses. MOH provided supervision to research assistants in assessments measures. IQ, BD and DH wrote the initial draft and all authors contributed to the final submission. HAN, FPM, STN, BR, SQB undertook screening and assessment measures and contributed to manuscript preparation.

**Data Sharing**

Individual participant data collected during the trial is available after de-identification immediately after publication and will end five years after article publication. Data requestors will need to complete a data access agreement and requests sent to inti.qurashi@nhs.net.

**Declaration of interests**

IBC reports giving lectures or advice to Eli Lilly, Bristol Myers Squibb, Lundbeck, AstraZeneca, and Janssen pharmaceuticals for which he or his employing institution have been reimbursed, outside the submitted work; IBC was previously trustee of the Pakistan Institute of Living and Learning (PILL). NH has been a past Trustee of the PILL, Abaseen Foundation UK, Lancashire Mind UK and Manchester Global Foundation (MGF). He is an executive member of the Academic Faculty at the Royal College of Psychiatrists, London. He is a NIHR Senior Investigator. He is director of research and innovation at Mersey Care NHS Foundation Trust. NH/IBC/AB & TK has attended educational events organized by various pharmaceutical industries. IQ is associate medical director of research and innovation at Mersey Care NHS Foundation Trust.

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