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Therapeutic effect of adjunctive *N*-acetyl cysteine (NAC) on symptoms of chronic schizophrenia: A double-blind, randomized clinical trial

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ABSTRACT

Background: Schizophrenia is one of the most disabling psychiatric syndromes with the prevalence of 1% in the general population. Despite availability of various antipsychotics, negative symptoms and cognitive impairment are difficult to treat. In addition antipsychotic monotherapy is not effective in most of these patients. Current evidence indicates the roles of glutamatergic system in this disorder. *N*-acetyl cysteine (NAC) also increases extracellular glutamate. This study was conducted to evaluate the clinical effects of oral NAC as an add-on to maintenance medication for the treatment of chronic schizophrenia.

Materials and methods: This 12-week, double-blind, randomized, placebo-controlled, clinical trial was performed to determine the effectiveness of 1200 mg *N*-acetyl cysteine as an adjunctive treatment with conventional antipsychotic medications in 84 patients with chronic schizophrenia. The subjects were evaluated with the Positive and Negative Syndrome Scale (PANSS), Mini-Mental State Examination (MMSE), and a standard neuropsychological screening test. Data were analyzed with SPSS-16 software.

Results: NAC-treated patients showed significantly improvement in the positive ($F = 5.47$, $P = 0.02$) and negative ($F = 0.20$, $df = 1$) PANSS subscale. Also the general and total PANSS score of NAC group declined over times whilst it was increased for placebo group. Regarding cognitive functions, improvement was observed in some explored areas, such as attention, short-term and working memory, executive functioning and speed of processing. There was no significant difference between the 2 groups in the frequency of adverse effects.

Conclusion: The present study detected improvement in positive, negative, general and total psychopathology symptoms as well as cognitive performance with NAC treatment. It is also well-tolerated, safe and easy-to-use agent as an effective therapeutic strategy to improve outcome in schizophrenia treatment.

1. Introduction

Schizophrenia is one of the most disabling psychiatric syndromes with the prevalence of 1% in the general population, the pathophysiology and etiology of this disorder has remained unknown (Sadock et al., 2009; Seeman, 2007; Sadock and Sadock, 2007). Schizophrenia increases the morbidity and mortality risk and health care costs (Wu et al., 2005). One of the considered mechanisms in etiology of schizophrenia is the defect in antioxidant system that causes rise of lipid peroxides level. Oxidative stress causes membrane defects, immune system dysfunction and pathology of different systems of the neurotransmitters in schizophrenia (Yao and Keshavan, 2011). On the other hand, in addition to the dopaminergic_dysfunctions in schizophrenia, other systems of neurotransmitters are engaged including GABA, serotonin, acetylcholine, noradrenalin (NA) and glutamate. Glutathione system dysfunctions have been illustrated in schizophrenia. Glutathione level, in these patients, decreases in blood, cerebrospinal fluid, and prefrontal cortex (Bloch, 2009; Marek and Behl, 2010). There is a 27% reduction in the cerebrospinal fluid levels of GSH in untreated patients with schizophrenia (Do et al., 2000) and 41% reduction in the caudate postmortem of schizophrenic patients (Yao et al., 2006). Previous studies recorded a significant decrease in the blood levels of total glutathione (Pavlovic et al., 2002 and Gawryluk, 2011) and significant decreased plasma levels of glutathione levels in drug-naive first-episode patients with schizophrenia (Raffa et al., 2011). In addition, the pathophysiology of the negative symptoms associated with the cognitive defects is still unknown (Dickinson and Harvey, 2009). Despite availability of various antipsychotics, negative symptoms and cognitive impairment are difficult to treat. Antipsychotic monotherapy is not effective in most of

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such patients and there have been still many health problems, especially in the treatment of negative symptoms as well as cognitive dysfunctions. Cognitive impairment represents a core feature of schizophrenia in the majority of patients with the disorder (Lewis, 2004). Cognitive functions have been shown to be associated with medication adherence and are the strongest predictors of patients' ability to manage medications (Jeste et al., 2003). The use of new pharmacological compounds on the treatment of schizophrenia has been suggested (Harrison et al., 2001; Hamer and Haddad, 2007). Furthermore alternative treatments, including anti-oxidant therapy has been considered in the treatment of patients with schizophrenia (Bondy and Spellmann, 2007; Reddy and Reddy, 2011; Bitanihirwe and Woo, 2011). *N*-Acetyl cysteine (NAC), an acetylated derivative of amino acid L-cysteine that is quickly absorbed orally, is a GSH precursor with antioxidant, neurotropic and anti-inflammatory properties, as well as modulatory effects on dopaminergic and glutamatergic systems (Arakawa and Ito, 2007). NAC acts effectively in the regulation of dopamine and glutamate neurotransmitter systems involved in schizophrenia (Olive et al., 2012) leading to regulate the synthesis of cysteine, releasing glutamate in the synaptic level and stimulating *N*-methyl-D-aspartate (NMDA) receptor (Steullet et al., 2006; Gere-Paszti and Jakus, 2009). There is increasing evidence that they converge on a common pathological hub that involves NMDA receptor hypofunction and oxidative stress in Schizophrenia (Hardingham and Kim, 2016(, GSH is depleted during oxidative stress, which can be reversed with NAC treatment (Atkuri et al., 2007).In vitro and in vivo studies have shown that NAC acts as cysteine prodrug and GSH precursor (Zafarullah et al., 2003). Also, the antioxidant role of NAC has been known in previous studies through_ regulating the synthesis of glutathione which decreases in schizophrenia (Altuntas et al., 2000: Gawryluk, 2011; Dodd et al., 2008). NAC has been effective in reverse oxidative stress associated with_ mitochondrial dysfunction (Wang et al., 2009; Otte et al., 2011).Another potential effect of NAC is the anti-inflammatory role that prevents the production of cytokines, leads to increase of glutamate, and regulates oxidative stress (Csontos et al., 2012; Tsai et al., 2009; Kigerl et al., 2012).

In a double-blind, randomized, placebo controlled trial on 140 patients with chronic schizophrenia, 69 patients treated with 2000 mg/day, NAC during 6 months had significant improvement in negative symptoms (Berk et al., 2008). Another study showed that patients with schizophrenia had significant improvement in the social interactions, motivations, psychomotor stability and behavior stability after treatment with NAC (Berk et al., 2011). In a small study, 11 patients with schizophrenia had significant reduction in the potential of auditory stimulation that was recorded by Mismatched negativity (MMN) after 8 weeks treatment with NAC. (Lavoie et al., 2008). A case report demonstrated significant improvement in symptoms after 600 mg daily dose of NAC for 4 weeks in a young woman with treatment-resistant schizophrenia (Bulut et al., 2009). Finally in a randomized, double-blind, placebo controlled study, 42 patients with chronic schizophrenia received (2000 m g/day) NAC during 2 months; as a result, NAC-treated patients showed significantly greater improvement in the total and negative symptoms (Farokhnia et al., 2013). All the above mentioned studies investigated the effect of 2000 mg/day dose of NAC on symptoms of schizophrenia. There has been no study to investigate the effect of other doses of NAC on symptoms of schizophrenia. Therefore, we attempted to investigate the ability of the lower dose of NAC (1200 mg daily) in increasing the effect of anti-psychotic drugs on the positive and negative symptoms and some areas of cognitive impairment of schizophrenia.

2. Methods

2.1. Trial design

This study was conducted as a twelve week, double blind randomized, placebo controlled, clinical trial. The study was performed on outpatients referred to Rehabilitation center of Kargarnejad psychiatric hospital in Kashan, affiliated with Kashan University of Medical Sciences (KUMS) from July 2015 to July 2016. The project was approved by the research and Medical Ethics Committee of Kashan University of Medical Sciences and carried out in agreement with declaration of Helsinki and its subsequent revisions. After complete explanation of the study details, written informed consent was obtained from the patients and legally authorized representatives in accordance with the procedures defined by the local Institutional Review Board. The participants were informed that they were free to withdraw from the study at any time. This trial was registered at the Iranian Clinical Trials Registry (IRCT:2015080223463 N1 www.irct.ir)

2.2. Participant

Patients who met the diagnostic criteria for schizophrenia based on the Diagnostic and Statistical Manual (DSM)-IV-TR diagnosed with chronic schizophrenia and were recruited for this study.

Patients were male or female with aged 18 to 65 years. The diagnosis was established by means of chart review and semi-structured clinical interview for DSM-IV-TR Axis I disorders (SCID). The patients were included if they (1) met DSM-IV-TR criteria for schizophrenia, (2) had minimum Positive and Negative Symptoms Scale (PANSS) score of 55 (3) had disease duration of at least 2 years (4) treat with chlorpromazine equivalent to 300–1000 mg (except clozapine) and anticholinergic agents equivalent to 4–8 mg Trihexyphenidyl during the 6 months prior. One hundred patients were screened for the eligibility criteria, 16 excluded before run in study and 84 patients were randomized into 2 groups. Two patients from NAC group and three patients from Placebo group dropped out from the trial because of either withdrawal of consent. Fig. 1 shows flow chart of study.

2.3. Exclusion criteria

Patients were excluded if they had any of the following criteria: (1) additional axis I diagnosis according to the DSM-IV-TR, (2) other neurologic or organic illnesses based on clinical examinations or laboratory findings, (3) Intelligent Quotient (IQ) of < 70 (mental retardation) based on clinical judgment, (4) any alcohol or substance dependence (except nico tine or caffeine) within past 6 months of screening, (5) score of ≥ 14 on a 17-item Hamilton Depression Rating Scale (HDRS) or a score of ≥ 4 on depression item of PANSS since significant depression can cause misinterpretation of the negative symptom,(6) treatment with clozapine, lithium, sodium valproic acid, carbamazepine, (7) presence of liver, kidney, thyroid, hematologic diseases and any serious chronic physical illness, (8) pregnancy or lactation, (9) patients who have recently received the known glutathione reduction inhibitor (like selenium or vitamin E), (10) the use of antidepressants including tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors in recent months,(11) previous allergic reaction to *N*-acetyl cysteine or any combination that includes the *N*-acetyl cysteine, (12) the history of seizure s, stroke and traumatic brain injury, (13) Suicidal ideation based on clinical judgment.

2.4. Interventions

After arriving the participants to the study, a number was assigned to each person and the participants randomized into two groups by Permuted Blocked Randomizations (each group n = 42).

All patients have been received antipsychotic (except clozapine) equivalent 300 to 1000 mg chlorpromazine from 6 months ego. After dividing participants were equally randomized into 2 groups, case group received 600 mg, two times a day (1200 mg/day) NAC (Hexal Pharmaceuticals, Holzkirchen, Germany) and placebo group received placebo (Barij Essence, Kashan, Iran). There was not any changes in dosages and type of prescribed therapy during study period.

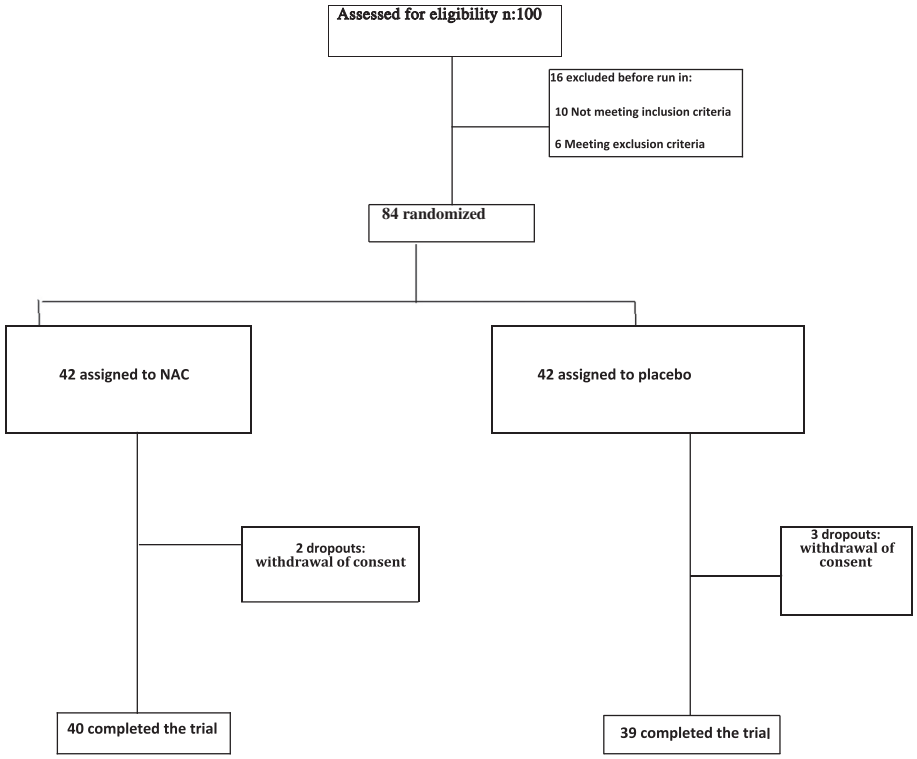


Fig. 1. Flow diagram of the trial.

2.5. Outcomes

Severity of psychiatric symptoms was assessed with Positive and Negative Symptoms Scale (PANSS), Neurocognitive function was evaluated via assessing changes in several domains of cognitive functioning. The Mini-Mental State Examination (MMSE), the Digit Span Forward and Backward Test, the Digit Symbol Substitution Test (DSST) and the Stroop Color-Word Test were used to assess cognitive function. These tests widely used in the study of cognition in schizophrenia. Patients attended in five visits: initial screening and randomization (week − 1), and four further visits at the weeks of 0, 4, 8, 12. Data for clinical and neurocognitive assessments were collected at weeks 0, 4, 8 and 12.

Measuring devices in this research was PANSS test made by Kay and colleagues as a means of measurement. The PANSS is a 30-item rating scale consisting of validated subscales to examine positive (7 items), negative (7 items), and general psychopathological (16 items) symptoms of schizophrenia. These 3 subscales are summed up in the PANSS total score (Kay et al., 1987). In Iran, the questionnaire was standardized (Bakhshipour Roudsari and Dejkam, 2005) and the PANSS test has been used in several clinical trials of schizophrenia in the Iranian population (Rezaei et al., 2013; Nikbakhat et al., 2016).

The following cognitive tests were administered at baseline and 12 weeks.

Adverse Events and safety thorough physical examination and ECG were performed at screening and each visit. All patients were encouraged to inform their health care providers and the research team about any unexpected adverse events at any time during this project. In order to provide more safety, open-ended questioning about any probable side effects were carried out during each visit followed by completion of a systematic 25-item questionnaire checklist of a broad range of alarming symptoms or complaints. Extra pyramidal side effects were also evaluated with physical examination at baseline and all post-baseline visits. The behavior appraisal and adverse effects checklist were completed by independent raters. In case of facing any side effect, an expert psychiatrist was responsible for deciding whether the patient was eligible to continue with the same drug dose, to continue with reduced dose or to discontinue.

2.6. Randomization and blinding

Randomization assignment was done using computer-generated random numbers and by a trained staff at the clinic as blindness. Allocation concealment was done using sequentially numbered, sealed, opaque packages. Both randomization and allocation were carried out by independent persons who were not involved elsewhere in the study. Patients, nurses, and physicians responsible for referring the patients, and the statistician, as well as the investigators who rated the patients and administered the drugs, were all blinded to the allocation. Placebo tablets were identical to NAC tablets in shape, odor, size, and color. They were all kept in identical containers and were administered by an investigational drug pharmacist.

2.7. Statistical analysis

IBM SPSS Statics version 20 (IBM Corporation) was used for data analysis. Continuous variables are reported as mean (SD) and categorical variables as number (percent). Mean differences (MD) are described with 95% confidence intervals [MD (95% CI)]. Independent sample *t*-test was used in order to compare the mean score changes of PANSS between the two groups at baseline and trial endpoint. ANOVA with repeated measures was employed to evaluate the interaction of time X treatment for PANSS scores between the two groups, assuming the study groups (NAC vs. placebo) as the between-subject variable and study measurements as the within-subject factor (time). In case of significant Mauchly's test of sphericity, Greenhouse-Geisser correction for degrees of freedom was performed. Statistically significant P-value was defined as < 0.05.

3. Results

3.1. Participants

One hundred patients were screened for the eligibility criteria and 84 patients were randomized into 2 groups. Two patients from NAC group and three patients from Placebo group dropped out from the trial

Table 1
Summary of neuropsychological tests.

Cognitive tests	Description	References
Digit Symbol Substitution Test (DSST) from the Wechsler Adult Intelligence Scale-Revised (WAIS-R)	A test assesses attention and speed of Processing. It consists of digit-symbol pairs followed by a list of digits. Each numeral (1 through 9) is associated with a different simple symbol. Subjects are required to copy as many of the symbols associated with the numerals as possible in 90 s. The number of correct symbols within the allowed time is measured.	Wechsler, 1981 Sumiyoshi et al., 2003
Digit span test from the Wechsler Adult Intelligence Scale-Revised (WAIS-R)	A test assesses short-term and working memory. Two digit sequences of the same length are presented at each stage.	Turner et al., 2003 Wechsler, 1981
Stroop Color and Word Test (SCWT)	A test to assess the ability to inhibit cognitive interference that occurs when the processing of a specific stimulus feature impedes the simultaneous processing of a second stimulus attribute, well-known as the Stroop Effect. In the Color Task, the individual reads aloud a list of color names in which no name is printed in its matching color. In the Color-Word Task, the individual names the ink color in which the color names are printed.	Trenerry et al., 1989 Scarpina and Tagini, 2017
Mini-Mental State Examination–(MMSE)	MMSE is a tool that can be used to assess mental status. It assesses orientation, registration, attention and calculation, recall, and language. The maximum score is 30. A score below 25 suggests possible impairment.	Crum et al., 1993 Seyedian et al., 2008

because of either withdrawal of consent. A total number of 79 patients (placebo, 39; NAC, 40) completed the trial (Fig. 1).

To get assurance about patient compliances for administered drugs interviewed with family members of patients. Respect to it, all patients used prescribed drugs according with psychiatric orders. Demographic characteristics of both groups were compared through *t*-test, Chi square and Fisher's Exact Test methods. There was not significant difference (P = 0.05), except for residential place variable. The mean age was 39.41 ± 13.95 and 38.75 ± 12.50 for treatment and placebo group respectively. Other details are presented in Table 2. (See Table 1.)

The baseline score of PANSS subscales were compared between two groups, using t-test analysis. The results are presented in Table 3. As it is shown there were significant differences between two groups. Therefore it was required to adjust baseline score across follow up period times.

3.2. Outcomes

To get insight about the effect of drug across measurement times and adjust of baseline score of PANSS differences, the repeated measure analysis was applied, whilst adjusting the residential places the covariate variable.

After checking repeated measure prerequisite assumptions with Mauchly and box test the PANSS subscale scores were compared within and between study groups across measurement times (at the baseline, 4th, 8th and 12th weeks).

Table 2
Demographic characteristics of study participants.

Variable		Study group		P-value
		NAC	Placebo	
Age (mean; SD)		38.7 (1.9)	39.4 (2.2)	n. s[a]
Duration of illness (mean; SD)		13.8 (9.9)	17(11.6)	n. s
Type of antipsychotic drug	Typical	10 (25.0)	13 (33.3)	n. s
	Atypical	27 (67.5)	23 (59.0)	
	Typical + Atypical	3 (7.5)	3 (7.7)	
Gender (%)	Male	18 (45)	20 (51.3)	n. s
	Female	22 (55)	19(48.7(	
Residential place (%)	Urban	26 (65)	17 (43.6)	P < 0.05
	Rural	14 (35)	22 (56.4)	
Education (%)	Illiterate	3 (7.5)	7 (17.9)	n. s
	Elementary	15 (37.5)	18 (46.2)	
	Intermediate	8 (20.0)	10 (25.6)	
	Diploma	9 (22.5)	2(5.1)	
	High educated	5)12.5)	2(5.2)	
Job (%)	Household	16 (40.0)	10 (25.6)	n. s
	Unemployed	20 (50.0)	26(66.7)	
	Employed	2 (5.0)	1(2.6)	
	Others	2 (5.0)	2 (5.1)	
Marital status (%)	Single	19 (47.5)	14 (35.9)	n. s
	Married	10 (25.0)	15 (38.5)	
	Widow/divorced	11 (27.5)	10 (25.7)	

[a] n. s; Non significant.

Table 3
Baseline PANSS score.

PANSS subscales	Study group		P-value
	NAC (SD)	Placebo (SD)	
Positive	22.9 (10.8)	17.7 (5.8)	P < 0.01
Negative	30.4 (7.3)	26.5 (6.2)	P < 0.05
General	50.5 (14.0)	43.5 (10.6)	P < 0.05
Total	104.0 (27.0)	87.7 (17.4)	P < 0.01

3.3. The PANSS positive subscale

Respect to repeated measure analysis results with applying Greenhouse-Geisser correction, there was not within groups significant difference over time (F = 1.2, df = 2.25, P = 0.31), however there was between groups significant differences (F = 5.47, df = 1, P = 0.02). In addition, there was interaction effect between time and group variables (F = 3.30, df = 2.25, P = 0.02). In other words, the reduction of PANSS positive score for NAC group was significantly greater than placebo group.

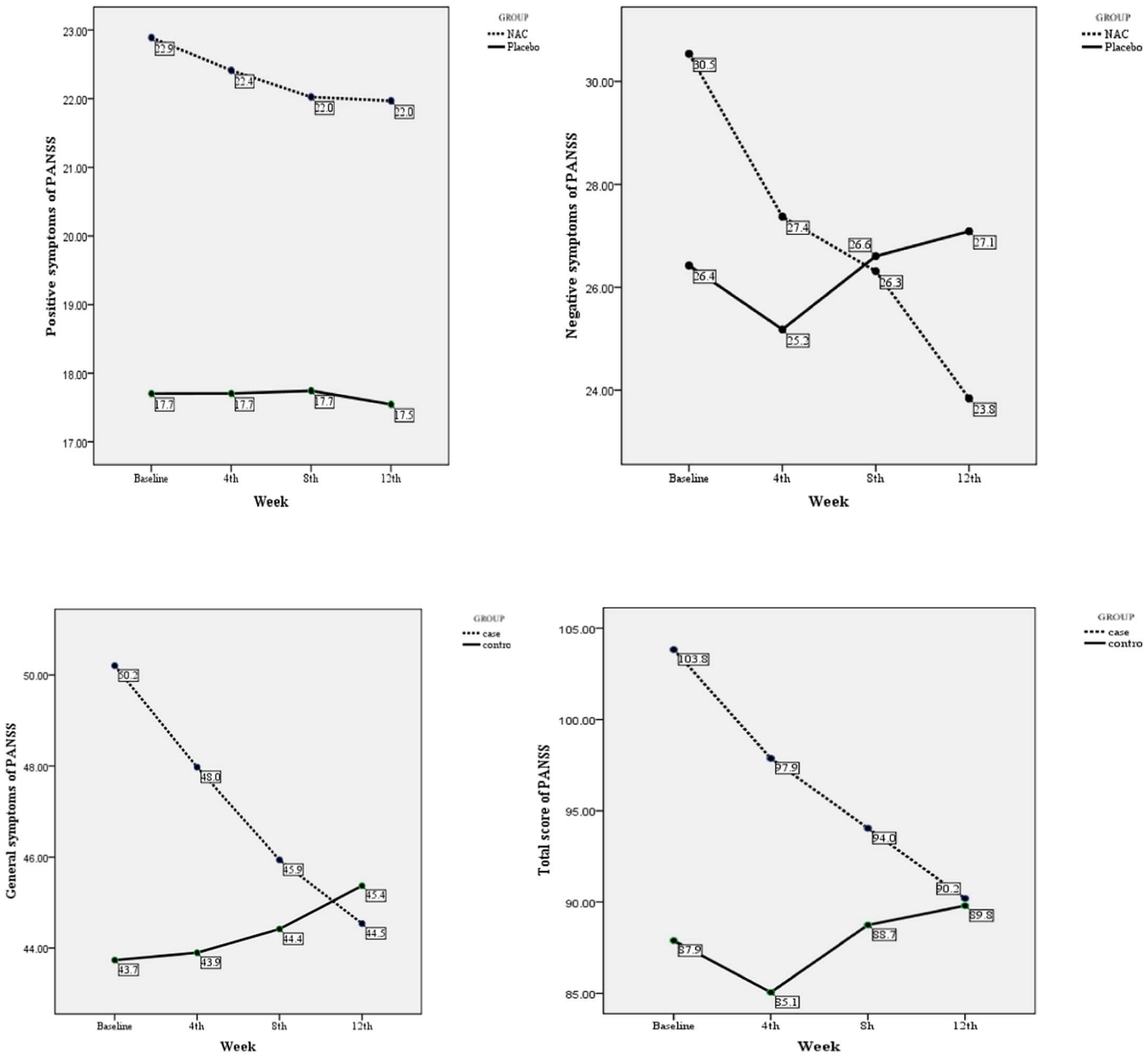
3.4. The PANSS negative subscale

For negative PANSS score with applying Huynh-Feldt correction, the PANSS negative score within study groups changed over time, significantly(F = 10.49, df = 2.56, P < 0.001). In between group comparison there was significant difference between NAC and placebo groups (F = 0.20, df = 1, P = 0.65).There was interaction between group and time (F = 27.79, df = 2.56, P < 0.001). The negative PANSS score in NAC group reduced gradually whilst for placebo group it was increased.

3.5. The PANSS general psychopathology subscale

With applying Huynh-Feldt correction, the general symptoms did not change over time (F = 4.52, df = 2.34, P < 0.01).

The mean general symptoms score of PANSS significantly decreased in the NAC group (P < 0.001) whilst for placebo group it was



Graph 1. The subscales changes of PANSS score within and between study groups during follow-up.

increased (P = 0.031). Compared to placebo group the effect of NAC on general symptoms improved over times. It means interaction between time and administered drug (F = 24.59, df = 2.34, P < 0.001).

3.6. The PANSS total subscale

For total PANSS score, the score changed over time, significantly (F = 9.10, df = 2.54, P < 0.001). The changes of the PANSS total score over times for study groups were different (F = 31.57, df = 2.54, P < 0.001). The total PANSS score of NAC group declined over times whilst it was increased for placebo group.

Graph 1 shows trend of changes of PANSS scores respect to study group.

3.7. Cognitive functioning assessment

Table 4 summarizes the results of the tests of cognitive functioning for both groups.

The mean MMSE score increased from 23.1 ± 4.4 at base line to 25.55 ± 15.4 at week 12 in the NAC group and from 23.79 ± 3.8 at baseline to 24.15 ± 3.76 at week 12 in the placebo group. However, these increases were significantly different at the end of study period (P < 0/001).

The mean Digit Span test (Forward) score increased from 6.2 ± 2.8 at base line to 7.9 ± 3.38 at week 12 in the NAC group and decreased from 5.76 ± 2.6 at baseline to 5.66 ± 2.59 at week 12 in the placebo group. However, there was significant difference between both groups (P < 0/001).

The mean Digit Span test (backward) score increased from 3.47 ± 1.8 at base line to 5.35 ± 2.79 at week 12 in the NAC group and decreased from 3.28 ± 1.65 at baseline to 3.23 ± 1.54 at week 12 in the placebo group. However, the difference between both groups remained significant (P < 0/001).

The mean Digit Symbol Substitution Test score increased from

Table 4
The effects of NAC compared with placebo on measures of cognitive functioning over the 12-week study period.

Cognitive assessment	NAC (N = 40) Mean ± SD		Placebo (N = 39) Mean ± SD		
	Baseline	12 weeks	Baseline	12 weeks	P-value
MMSE*	23.1 ± 4.4	25.55 ± 15.4	23.79 ± 3.8	24.15 ± 3.76	< 0/001
Digit Span test					
Forward	6.2 ± 2.8	7.9 ± 3.38	5.76 ± 2.6	5.66 ± 2.59	< 0/001
Backward	3.47 ± 1.8	5.35 ± 2.79	3.28 ± 1.65	3.23 ± 1.54	< 0/001
DSST	17.37 ± 9.8	25.07 ± 11.9	27.92 ± 14.84	27.84 ± 15.15	0/003
Stroop test (Letter)					
	37.32 ± 4.8	35.07 ± 5.45	37.74 ± 5.25	38.53 ± 3.5	< 0/001
Stroop test (Color)	31.79 ± 3.83	29.55 ± 5.08	32.10 ± 4.38	32.74 ± 4.19	< 0/001

Data were analyzed using repeated-measures analysis of variance (ANOVA). P-value was calculated from time by group interaction. MMSE: Mini Mental State Examination, SD: standard deviation, DSST: Digit Symbol Substitution Test.

17.37 ± 9.8 at base line to 25.07 ± 11.9 at week 12 in the NAC group and decreased from 27.92 ± 14.84 at baseline to 27.84 ± 15.15 at week 12 in the placebo group. Although, there was significant difference between both groups (P = 0/003).

The mean stroop test (letter) score decreased from 37.32 ± 4.8 at base line to 35.07 ± 5.45 at week 12 in the NAC group and increased from 37.74 ± 5.25 at baseline to 38.53 ± 3.5 at week 12 in the placebo group. Even so, the difference between both groups stayed significant (P < 0/001).The mean stroop test (color) score decreased from 31.79 ± 3.83 at base line to 29.55 ± 5.08 at week 12 in the NAC group and increased from 32.10 ± 4.38 at baseline to 32.74 ± 4.19at week 12 in the placebo group. However, these increases were significantly different at the end of study period (P < 0/001).

3.8. Adverse effects and safety

There were neither significant effects of 1200 mg NAC on safety parameters, no reported adverse events by the participants. Only one patient in NAC group reported the mild abdominal cramp and discomfort, this adverse effect, did not result in discontinuation of the drug intake.

4. Discussion

In this study, we found significant reduction in the mean negative symptoms score, the positive symptom score, the general psychopathology score and total score for the NAC group compared with the placebo group by the study endpoint. We also found a significant time × treatment interaction for all PANSS scores.

The reduction of PANSS positive score for NAC group was significantly greater than placebo group. In a similar study by Berk et al. conducted on 140 patients with chronic schizophrenia in 2008, 84 patients were treated by with 2000 mg per day NAC for 6 months, as adjuvant therapy of antipsychotics and no significant changes in scale of PANSS test positive symptoms were seen (Berk et al., 2008). In another double-blind study performed in 2013 by Farokhnia et al. in Iran on 42 patients with chronic schizophrenia, the effect of the adjuvant therapy of NAC (2000 mg daily) to Risperidone (at least 6 mg per day) for 8 weeks on symptoms of schizophrenia were evaluated. They did not observed any significant effects of the drugs on the positive symptoms (Farokhnia et al., 2013). Therefore, the differences between the results of these studies on the improvement of positive symptoms with NAC therapy can be attributed to differences in studied populations or study designs or differences of doses. That warrant further investigation to be clarified. To date, any clinical trials have not investigated this dose of NAC on positive symptoms in patients with schizophrenia.

Also, for negative PANSS score there was significant difference between the two groups and the administered drug effects on negative PANSS score changed significantly over time. The results of this study are similar to the results of the study performed by Berk et al. in 2008 (Berk et al., 2008) and Farokhnia et al. in 2013 (Farokhnia et al., 2013). Therefore, this study showed that lower doses (1200 mg) of NAC have been prescribed compared to other studies with higher dose (2000 mg daily) is effective on negative symptom of schizophrenia. This dose is safer and cost-benefit.

About general symptoms there was significant difference between the study groups. The PANSS mean score of the general symptoms significantly decreased among the patients studied in the NAC group. Whilst, the PANSS mean score of the general symptoms significantly increased among the patients studied in the placebo group. The results of this study were similar to the results of the study performed by Berk et al. in 2008 (Berk et al., 2008) in which the general symptoms of the schizophrenic patients in the NAC group had significant improvement compared to the placebo group (Berk et al., 2008) but our results were opposite of the study of Farokhnia et al. in 2013 (Farokhnia et al., 2013) in which was not determined any significant difference in the reduction of PANSS general psychopathology subscale scores. Therefore, the differences between the results of the 2 studies on the improvement of general symptoms with NAC therapy can be attributed to different doses of the drug, the methodology the study population, the study design or more sample size in our study and longer observational period in our study (12 weeks comparison with 8 weeks) that warrant further investigation to be clarified.

Also, for total PANSS score there were significant difference between study and placebo group. The mean score of the patients studied in the group receiving NAC has been significantly decreased. While, the score mean of the total symptoms PANSS of the patients in the group receiving the placebo has been increased at the end of the study. The results of this study are similar to the results of the study performed by Berk et al. in 2008 (Berk et al., 2008) and Farokhnia et al. in 2013 (Farokhnia et al., 2013) on the total symptoms of the patients (Farokhnia et al., 2013). Therefore, this study showed that lower doses (1200 mg) of NAC have been prescribed compared to other studies with higher dose (2000 mg daily) is effective on total symptom of schizophrenia too. This dose is safer and cost-benefit.

In the present study, NAC enhanced speed of processing and attention on the DSST, whilst in the patients given placebo at 3 months, slight deterioration was noted in the speed of processing. In a double-blind, randomized, placebo-controlled trial in 63 schizophrenic patients, they assessed the effect of NAC supplementation (2700 mg/day, 6 months) on PANSS, neurocognition, and redox markers (brain GSH, blood cells GSH levels, GSH peroxidase activity). No changes in negative or positive symptoms or functional outcome were observed with NAC, but significant improvements were found in favor of NAC on neurocognition (processing speed). NAC improved the processing speed

(PS) factor containing these tasks (Conus et al., 2017). A study by Keefe et al. in 2006, they reported the neurocognitive data from patients who participated in the CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness) Schizophrenia trial. By analyzing data from a comprehensive test battery, authors have found that the DSST by itself accounted for the most of the test variance (Keefe et al., 2006). Performance on the DSST represents attention and processing speed. This neurocognitive impairment has been found to be correlate with a variety of clinically important features of schizophrenia, such as daily life activities, job tenure, and independent living status (Sadock and Sadock, 2007). Therefore this study indicated that adjunctive *N*-acetyl cysteine therapy has beneficial effects on speed of processing and attention. There is ample evidence to suggest that increased levels of oxidative stress, inflammation, mitochondrial dysfunction and apoptosis are associated with clinically meaningful cognitive deterioration (Skvarc et al., 2017). NAC can be protects against oxidative stress and is likely neuroprotective demonstrating pre-clinical efficacy in reducing markers of oxidative stress and the severity of cognitive dysfunction in animal models (Hsiao et al., 2012; Huang et al., 2010). Similar oxidative responses have been detected in humans (Moreira et al., 2007). Supplementation of NAC can be effective in reducing the severity of cognitive changes associated with a variety of disorders characterized by oxidative stress (Skvarc et al., 2017).

Significant improvements were observed in performance on short-term and working memory, as measured by the digit span test. In addition, NAC significantly improved attention and executive functioning on the stroop test. Finally significant improvements in the mean MMSE score were observed. In study of Rapado-Castro et al. in 2016, 58 participants with Psychotic disorders (both affective and non-affective disorders) received 2 g/day of NAC for 24 weeks. Attention, working memory and executive function domains were assessed. They reported NAC results in a significant improvement only in working memory in the NAC-treated group (Rapado-Castro et al., 2017). The differences between the results of the 2 studies on the improvement of attention and executive function with NAC therapy in our study can be attributed to differences in studied population, all of participants in our study were patients with diagnosis of schizophrenia but in study of Rapado-Castro et al., patients with Psychotic disorders (both affective and non-affective disorders), were included. That warrants further investigations to be clarified. Other study that evaluated the effect of NAC on symptom of schizophrenia, did not investigated the effect of NAC on cognitive impairment (Berk et al., 2008; Farokhnia et al., 2013; Lavoie et al., 2008 and Bulut et al., 2009). Therefore we had no prior data to calculate base power.

N-Acetyl cysteine appears to be promising in the treatment of schizophrenia. There are several reasons to explain why NAC can be helpful in treating schizophrenia. *N*-Acetyl cysteine (NAC) is derived from the amino acid L-cysteine (Arakawa and Ito, 2007). When NAC reaches brain glial cells, it is oxidized to cysteine, then enters the cells in exchange for glutamate and leads to increase extracellular glutamate. NAC acts effectively in the regulation of dopamine and glutamate neurotransmitter systems involved in schizophrenia (Olive et al., 2012) leading to regulate the synthesis of cysteine, releasing glutamate in the synaptic level and stimulating *N*-methyl-D-aspartate (NMDA) receptor (Steullet et al., 2006; Gere-Paszti and Jakus, 2009). Also, the antioxidant role of NAC has been known in previous studies through regulating the synthesis of glutathione which decrease in schizophrenia (Altuntas et al., 2000; Gawryluk, 2011; Dodd et al., 2008). On the other hand, NAC has been effective in reverse oxidative stress associated with mitochondrial dysfunction (Wang et al., 2009; Otte et al., 2011). Another potential effect of NAC is the anti-inflammatory effect that prevents the production of cytokines, leading to increase glutamate and regulates oxidative stress (Csontos et al., 2012; Tsai et al., 2009; Kigerl et al., 2012). Glutamatergic receptors are among the most promising biological targets for cognitive-enhancing drugs in schizophrenia.

This trial showed that, 1200 mg of NAC is safer. To date, no clinical trial has investigated this dose of NAC on symptoms in patients with schizophrenia. Only one patient in NAC group reported the mild abdominal cramp and discomfort. This adverse effect did not result in discontinuation of the drug intake. Other study performed by Berk et al. in 2008 (Berk et al., 2008) and Farokhnia et al. in 2013 (Farokhnia et al., 2013) prescribed higher dose (2000 mg daily) of NAC, In clinical trial of Farokhnia et al. in 2013 (Farokhnia et al., 2013), some adverse effects such as drowsiness, constipation, dizziness, vomiting, increased appetite, nausea, headache, dry mouth were observed over the course of the trial on the basis of the adverse effects checklist. But we don't detected significant difference between the 2 groups in the frequency of adverse effect so it seems the dose of 1200 mg NAC is more safe and cost-benefit than 2000 mg/day NAC.

So, the results of this study support the effectiveness of oral NAC, 1200 mg daily, in reduction of the score of PANSS in the patients with chronic schizophrenia. Of course, improvement happened in negative, general and total symptoms and regarding cognitive functions, it was also observed in some explored areas, such as attention, short-term and working memory, executive functioning and speed of processing. The cognitive impairment and negative symptoms represent a cardinal feature of schizophrenia and are strong predictors of poor vocational and social outcomes. Our findings indicate that adjunctive *N*-acetyl cysteine therapy has beneficial effects on cognitive functioning or psychopathology among patients with schizophrenia.

The results of this trial include several limitations. Firstly, we tested only one dose of NAC.

Secondary, we could not evaluate a biomarker of glutathione status to gauge the biological effects of the NAC dose. Thirdly, this study had a relatively short duration of intervention. Finally, cognitive impairment evaluate only in some area of cognitive functioning. Despite these limitations, we found evidence of significant benefit. This trial may now be utilized for future studies. Additional studies with long-term follow-up periods and other dose of NAC seem to be essential to reinforce clinical use of adjuvant NAC in patients with schizophrenia. In addition, further studies that address the limitations described above is needed to confirm NAC effect on a wider range of area of cognitive functioning in schizophrenia.

5. Conclusion

The present study detected improvement in negative, general and total psychopathology symptoms as well as cognitive performance with NAC treatment. It is also well-tolerated, safe and easy-to-use agent as an effective therapeutic strategy to improve outcome in schizophrenia treatment.

Ethical statement

We reported this original research with accurate and sufficient detail data. We written entirely original work. We don't submit our research in another journal and this paper has not been published previously. We have met the confidentiality. The project was approved by the research and Medical Ethics Committee of Kashan University of Medical Sciences and carried out in agreement with declaration of Helsinki and its subsequent revisions. After complete explanation of the study details, written informed consent was obtained from the patients and legally authorized representatives in accordance with the procedures defined by the local Institutional Review Board. The participants were informed that they were free to withdraw from the study at any time. This trial was registered at the Iranian Clinical Trials Registry (IRCT:2015080223463N1 www.irct.ir).

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