RESEARCH

Investigation of the effect of *N*-acetylcysteine on serum levels of oxidative inflammatory biomarkers in patients with stroke

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Abstract

Background *N*-acetylcysteine (NAC) is a tolerable and safe drug capable of reducing free radicals and other oxidants. We included 74 individuals with ischemic stroke in this randomized, single-blind clinical trial and placed them into intervention ($n = 37$) and control ($n = 37$) groups. In the intervention group, in addition to standard treatment for ischemic stroke, they received NAC at a dose of 100 mg/kg bolus and then at a dose of 10 mg/kg/h for 10 h. The control group received only standard stroke treatment. Blood samples were taken before starting NAC and standard stroke treatment and 24 h after receiving the drug to measure the catalase, paraoxonase, malondialdehyde (MDA), neopterin, total antioxidant capacity (TAC), and total oxidant status (TOS) parameters. The National Institutes of Health Stroke Scale (NIHSS) was also calculated before and after 24 h, 2 weeks, 1 month, and 3 months after starting the drug.

Results There was no significant difference between the results of parameters before and after standard treatment in control group; however, NAC could significantly reduce TOS ($P = 0.02$) in the intervention group. Moreover, NAC administration could notably decrease NIHSS calculated at each time point when compared to control group. After subgrouping the intervention group, NAC could increase catalase ($P < 0.001$), paraoxonase ($P < 0.001$), and TAC ($P < 0.001$) while decreased MDA ($P < 0.001$), neopterin ($P = 0.001$) and TOS ($P < 0.001$) significantly in intervention-responding subgroup and decreased NIHSS significantly at each monitored time point.

Conclusion NAC can be promising as a complementary drug and a powerful antioxidant in reducing oxidative stress and improving cognitive function in individuals with stroke.

Keywords Stroke, Oxidative stress, *N*-acetylcysteine, Antioxidant biomarkers

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1 Background

Ischemic and hemorrhagic strokes have different risk factors and pathophysiological mechanisms, and however, there is evidence of an increase in free radicals in both pathologies, that leads to oxidative stress [1–3]. Cerebral ischemia (especially in the post-restorative period) is associated with the formation of reactive oxygen species (ROS) in brain tissue [4, 5]. Overproduction of ROS such as superoxide anion (O_2^-), hydroxyl radical (OH·), hydrogen peroxide (H_2O_2), and nitric oxide has been shown to play an important role in the development of ischemia/ reperfusion of blood flow [6, 7]. Oxidative stress leads to an increase in oxidative markers such as malondialdehyde (MDA) and neopterin and a decrease in the level of enzymes such as catalase and paraoxonase in individuals with stroke [8–11]. Antioxidant therapy is useful in suppressing nerve damage. There is ample evidence to support the beneficial effects of antioxidants such as lipoic acid, vitamin E, melatonin, and ebselen on ischemic brain injury and stroke [1, 12, 13]. *N*-acetylcysteine (NAC) which is commonly used as the antidote to acetaminophen in overdose scenarios is a tolerable and safe drug that effectively reduces free radicals and other oxidants [14–16]. It is a membrane-permeable cysteine precursor, that acts as the ROS scavenger. Additionally, NAC increases the intracellular cysteine pool; boosts the release of protein thiols through disulfide cleavage, therefore, escalating glutathione (GSH) levels. Moreover, NAC is capable of enhancing the GSH-dependent detoxification activity of H_2O_2 by antioxidant enzymes mainly glutathione peroxidase and thioredoxin [17]. NAC has been at the center of attention due to its effectiveness in some pathologies and diseases in humans such as the prevention of chronic obstructive pulmonary disease (COPD) exacerbation [18], prevention of contrast-induced nephropathy during the imaging process [19], improving the health condition in influenza virus infection, and improving cell-mediated immunity [20], and promising treatment of pulmonary fibrosis [21]. Previously, NAC has been reported effective in improving oxidative damage caused by ischemia and reperfusion in animal models. In this regard, post-cerebral ischemia–reperfusion administration of NAC has been reported to be protective and beneficial against free radical injury, and inflammation in rodent models [14, 22]. Although the effectiveness of NAC is proven in many settings, there is a paucity of data on the activity of NAC on oxidative stress biomarkers, serum level oxidative inflammatory biomarkers in individuals with stroke, hence in the present study, we used a panel of oxidative and inflammatory biomarkers and studied the effects of NAC in an intervention group of 37 individuals with ischemic stroke as a supplement (along with their standard treatment) against

a control group of the same number receiving only standard treatment. Moreover, as the second objective, from a clinical point of view, monitored the effects of NAC on NIHSS in four monitoring points (24 h, 2 weeks, 1 month, and 3 months) post-intervention.

2 Methods
2.1 Subjects
In this randomized clinical trial study (IRCT registration number: IRCT2017011931229N2), 74 individuals were recruited from the neurology ward in Firoozgar Hospital (Tehran-Iran) in 2021. We divided them into two intervention and control groups (each consisting of 37 individuals). The sample size with a confidence interval of 95% and power of 90% for average NIHSS was estimated to be 37 individuals in each group. To calculate the sample size, we referred to previous studies [23], used the Stata software (sd1 = 2.65, sd2 = 3.53, power = 0.90, alpha = 0.0500 (two-sided), m1 = 6.29, m2 = 8.9, n2 = 37/n1 = 37, n1 = n2 = 37). Moreover, we checked our results and confirmed them with the following equation. (Confidence level = $1 - \alpha$, power of test = $1 - \beta$)

$$n = \frac{(z_{1-\alpha/2}+z_{1-\beta})^2*(\sigma_1^2+\sigma_2^2)}{(\bar{x}_1-\bar{x}_2)^2}$$

The randomization was applied using the website www.sealedenvelope.com, and the participants of the intervention were assigned accordingly. A unique number was then produced and dedicated to each participant to divide them into two groups. The allocation sequence was given then to the person in charge of randomization in the clinic in a sealed form using envelopes containing the number of participants. (Fig. 1) In all participants, stroke was diagnosed based on neurological and clinical tests as well as magnetic resonance imaging (MRI). The intervention group received NAC in addition to standard treatment (aspirin 300 mg and clopidogrel 300 mg on the first day of stroke) for ischemic stroke at a dose of 100 mg/kg bolus 1 and then at a dose of 10 mg/kg/h for 10 h. Written consent was obtained from all individuals before the experiment. The protocols of this study are approved by the ethics committee of Kermanshah University of Medical Sciences (ethics code IR.KUMS.REC.1399.563).

2.2 Primary and secondary outcome measurement
2.2.1 Primary outcome measurement
MDA, neopterin, and TOS in recruited groups are expected to increase at primary investigation, while catalase, paraoxonase, and TAC to decrease on admission, we hypothesized NAC could reduce MDA, neopterin, and TOS but increase catalase, paraoxonase, and TAC, 24 h after being administered. Additionally, we studied
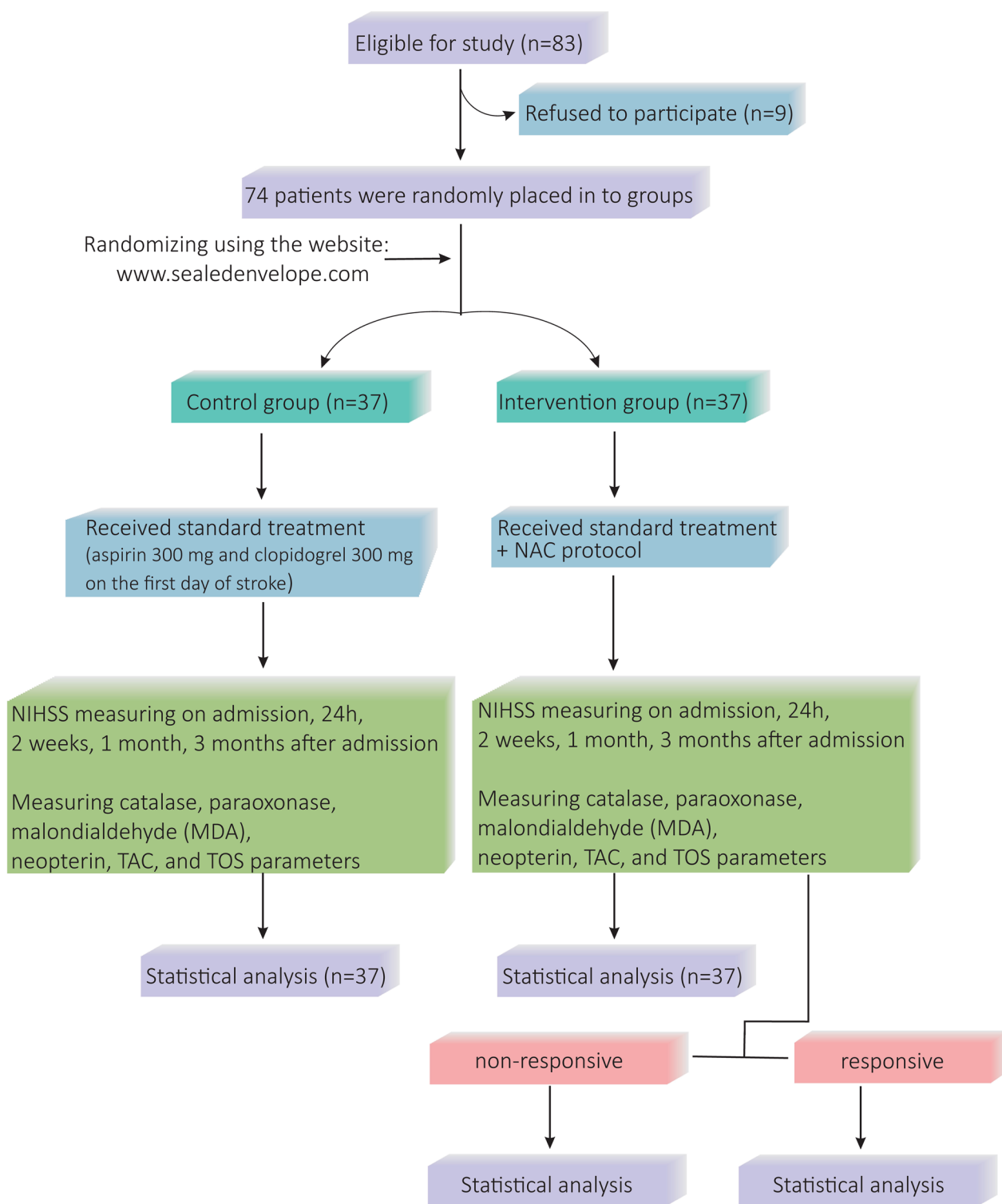
Fig. 1 Flowchart indicating the grouping process of individuals into intervention and control groups and the randomization process

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this panel in the intervention group after dividing it into intervention-responsive and non-responsive subgroups.

2.2.2 Secondary outcome measurement
NIHSS was calculated in both subgroups by a neurologist on admission day, 24 h, 2 weeks, 1 month, and 3 months after hospital admission. The effects of NAC administration on lowering NIHSS were monitored in the intervention group compared to control and then between intervention-responsive and non-responsive subgroups.

2.3 Blood collection and neurological examinations
Blood samples were collected from both groups before starting NAC and standard stroke treatment and 24 h after receiving the drug to measure the parameters including catalase, paraoxonase, MDA, neopterin, TAC, and TOC. NIHSS for individuals before the intervention, 24 h, two weeks, one month, and three months later was calculated by a neurologist for both groups.

2.4 Biochemical tests
2.4.1 Measurement of catalase activity
Catalase activity was measured according to the method of Sinha et al. [24]. In this method, acetic acid-soluble dichromate was reduced to chromic acid in the presence of hydrogen peroxide and heat. We used four tubes, control, test, standard, and blank to measure catalase activity. 25 µl serum and 250 µl of H_2O_2 were added to the test tube. In the control tube, 25 µl of serum and 250 µl water were added. In the standard tube, 25 µl of distilled water and 250 µl of H_2O_2 were added, while a tube containing 275 µl of distilled water was used as the blank tube. All tubes were gently shaken and incubated for 3 min at 37 °C. 500 µl potassium dichromate and glacial acetic acid were then added to each tube, and the tubes were gently shaken and placed in a boiling water bath (100 °C) for 10 min and left to gradually cool down. We then centrifuged all tubes at 2500 rpm/5 min, and the absorbance was measured at 570 nm using a spectrophotometer (PerkinElmer Lambda, USA), and the catalase activity was calculated using the equation used by Sinha et al. [24].

2.4.2 Measurement of paraoxonase-1 arylesterase activity
To measure paraoxonase-1 arylesterase activity, 5 µl of serum was added to 1 mM phenylacetate as the substrate in a buffer containing a mixture of 20 mM Tris–HCL and 1 mM $CaCl_2$ (pH = 8). Initial rates of hydrolysis were determined by monitoring the increase in phenol concentration at 270 nm at 37 °C and enzyme activity was calculated in IU / Liter in serum.

2.4.3 Measurement of Total antioxidant capacity (TAC)
Total antioxidant capacity was assessed by a commercial kit (KIAZIST Life Sciences, Iran). In this experiment, cupric (Cu^{+2}) is reduced to copper (Cu^{+1}) in the presence of oxidants that develops color in the presence of chromogen. The amounts of antioxidants are directly related to the amount of absorption. The absorbance was determined at 450 nm (PerkinElmer Lambda, USA).

2.4.4 Measurement of Total oxidative status (TOS)
To measure the total oxidant status, a commercial kit was used (KIAZIST Life Sciences, Iran). In this experiment, ferrous (Fe^{2+}) oxidizes to ferric (Fe^{3+}) in the presence of oxidants and produces color in the presence of chromogen. The amount of absorption is directly related to the amount of oxidant. The absorbance was determined at 560 nm [25].

2.4.5 Measurement of malondialdehyde (MDA)
In this study, 50 µl of plasma sample of each included individual was added to 50 µl of the 2,6-di-tert-butyl-4-methylphenol solution, 400 µl of the phosphoric acid solution, and 100 µl of 2-thiobarbituric acid (TBA) solution. The samples were then heated at 100 °C for 1 h, and then, the samples were placed in ice water for 5 min. After cooling the samples to extract the MDA-TBA derivative, 250 µl of n-butanol was added and vortexed for 5 min and then centrifuged for 5 min at 14,000 rpm. Finally, 20 µl of n-butanol supernatant containing MDA-TBA was injected into the HPLC device (HPLC-Agilent 1200, USA), and then, the amount of each sample was calculated using a standard curve. The fluorescence detector was set at an excitation wavelength of 515 nm and an emission wavelength of 553 nm [26, 27] (Fig. 2a).

2.4.6 Measurement of serum neopterin level
Serum neopterin levels were measured by the HPLC Agilent 1200, USA. For the neopterin analysis, 100 µL of TCA (5%) was added to 100 µL of standard or serum sample and vortexed for 10 s. The samples were centrifuged at 5000 rpm for 5 min. 50 µL of supernatant was diluted with 200 µL of distilled water and 20 µL was used for injection to HPLC for analysis and the wavelength of the fluorescence detector was set at 353 nm for excitation and 438 nm for emission [28] (Fig. 2b).

2.4.7 Statistical analysis
The collected data were analyzed using SPSS software version 16, IBM, USA. Qualitative data were described using the chi-square test as frequency percentage
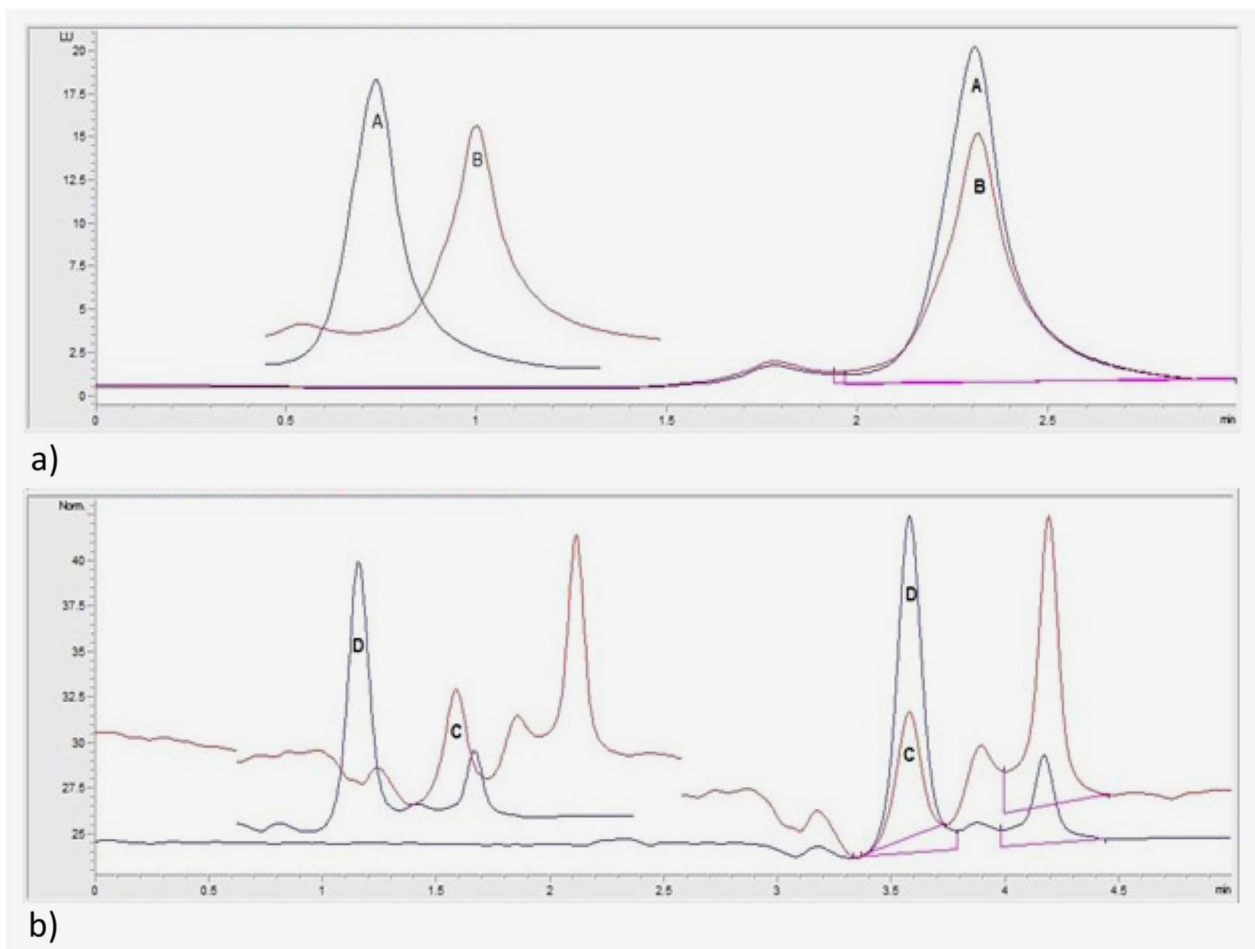
Fig. 2 a Chromatogram regarding MDA measurement; A: serum levels of MDA before intervention, B: serum levels of MDA after intervention (retention time = 2.4 min). The overlay chromatogram of both A and B is depicted on the right, while the 3D chromatogram is shown on left. **b** Chromatogram regarding neopterin measurement; C: serum levels of neopterin after intervention, D: serum levels of neopterin before intervention (retention time = 3.2 min). The overlay chromatogram of both C and D is depicted on right, while the 3D chromatogram is shown on left

and one-dimensional and two-dimensional tables, and quantitative data were described using mean and standard deviation. Compliance of variables with normal distribution was measured using the Kolmogorov–Smirnov test. When the normality condition was met, paired t test was used to compare the means before and after in each group; otherwise, the Wilcoxon test was alternatively used. Student t test was used to compare the means between the two groups when the normality condition was met; otherwise, Mann–Whitney test was used. The level of statistical significance was set at $p < 0.05$.

3 Results

A total of 74 individuals with stroke were included in this study and divided into intervention and control groups each consisting of 37 individuals. There were 20 (54%)

Table 1 Demographic information and clinical characteristics

Variable	Intervention group	Control group	*p* value
Age, (year)	62.48 ± 11.43	62.71 ± 12.11	0.94
Male, n (%)	20 (54.0)	25 (67)	0.16
Female, n (%)	17 (46.0)	12 (33)	
DM, n (%)	6 (19.35)	9 (29.03)	0.45
HLP, n (%)	3 (9.67)	6 (19.35)	0.27
HTN, n (%)	16 (51.61)	15 (48.38)	0.65
Smoking, n (%)	11 (35.48)	6 (19.35)	0.15
Glucose (mg/dl)	130.13 ± 38.43	146.13 ± 82.18	0.33
LDL (mg/dl)	115.63 ± 34.25	110.55 ± 34.87	0.64
HDL (mg/dl)	37.68 ± 9.25	40.60 ± 9.70	0.34

DM Diabetes mellitus, *HLP* Hyperlipidemia, *HTN* Hypertension, *LDL* Low-density lipoprotein, *HDL* high-density lipoprotein

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males and 17 (46%) females in intervention group, while the control group was consisting of 25 (67%) male and 12 (33%) female individuals. The mean age of the individuals in intervention group was 62.48 ± 11.43 years versus 62.71 ± 12.11 years in control group. There was no significant difference in age and gender between the two groups (*P* values 0.94 and 0.16, respectively). Investigation of their medical history showed no significant differences in clinical and laboratory test results and findings: *P* value$_{DM}$ = 0.45, *P* value$_{HLP}$ = 0.27, *P* value$_{HTN}$ = 0.65, *P* value$_{smoking}$ = 0.15, *P* value$_{glucose}$ = 0.33, *P* value$_{LDL}$ = 0.64, and *P* value$_{HDL}$ = 0.34 (Table 1).

Our results showed no significant difference between the studied parameters before and after receiving the standard treatment in control group:

$TAC_{control/before} = 3227.79 \pm 192.53$ versus $TAC_{control/after} = 3281 \pm 279.37$ (*P* = 0.82), $TOS_{control/before} = 74 \pm 9.10$ versus $TOS_{control/after} = 71.49 \pm 8$ (*P* = 0.83), $MDA_{control/before} = 11.84 \pm 1.26$ versus $MDA_{control/after} = 11.49 \pm 6.23$ (*P* = 0.78), $Catalase_{control/before} = 4.87 \pm 1.22$ versus $Catalase_{control/after} = 7.60 \pm 1.38$ (*P* = 0.14), $Paraoxonase_{control/before} = 21.97 \pm 1.61$ versus $Paraoxonase_{control/after} = 20.75 \pm 1.38$ (*P* = 0.55), and $Neopterin_{control/before} = 2.02 \pm 0.56$ versus $Neopterin_{control/after} = 0.97 \pm 0.14$ (*P* = 0.06). Although the overall trend showed that individuals in intervention group who received NAC showed improvement in antioxidant parameters after receiving NAC compared to results of preintervention, however, it was not significant, except for TOS.

$TAC_{Intervention/before} = 3474 \pm 257.48$ versus $TAC_{Intervention/after} = 3579.50 \pm 210.16$ (*P* = 0.75), $TOS_{Intervention/before} = 113.28 \pm 12.57$ versus $TOS_{Intervention/after} = 79.70 \pm 8.25$ (*P* = 0.02), $MDA_{Intervention/before} = 10.14 \pm 1.75$ versus $MDA_{Intervention/after} = 8.30 \pm 1.24$ (*P* = 0.42), $Catalase_{Intervention/before} = 4.44 \pm 0.68$ versus $Catalase_{Intervention/after} = 6.19 \pm 0.94$ (*P* = 0.10), $Paraoxonase_{Intervention/before} = 22.03 \pm 1.43$ versus $Paraoxonase_{Intervention/after} = 22.61 \pm 1.35$ (*P* = 0.55), and $Neopterin_{Intervention/before} = 1.81 \pm 0.36$ versus $Neopterin_{Intervention/after} = 1.54 \pm 0.27$ (*P* = 0.13).

When comparing control and intervention groups, we found no significant difference between the results of studied parameters except for TOS. The *p* values corresponding to each test are as following: TAC: *P* = 0.46, TOS: *P* = 0.02, MDA: *P* = 0.44, catalase: *P* = 0.75, paraoxonase: *P* = 0.97, and neopterin: *P* = 0.09.

Moreover, NIHSS showed an overall improvement during the studied time points until the first month, and its significance dropped down slightly in the 3rd month of intervention. (The higher the score, the more severe the stroke).

$(NIHSS/24h_{Intervention} = 4.38 \pm 0.99$ vs $NIHSS/24h_{control} = 6.82 \pm 1.24$, *p* = 0.134, $NIHSS/2weeks_{Intervention} = 2.94 \pm 0.70$ vs $NIHSS/2weeks_{control} = 5.93 \pm 1.36$, *p* = 0.05, $NIHSS/1month_{Intervention} = 2.22 \pm 0.56$ vs $NIHSS/1month_{control} = 6 \pm 1.63$, *p* = 0.02, and $NIHSS/3months_{Intervention} = 1.83 \pm 0.51$ vs $NIHSS/3months_{control} = 3.53 \pm 0.71$, *p* = 0.057). According to the provided data, the values corresponding to each period decreased in intervention group when compared to the controls. When comparing each NIHSS calculated in a specific period post-intervention to that of NIHSS calculated

Table 2 Comparison of NIHSS and mean serum levels of TAC, TOS, MDA, catalase, paraoxonase and neopterin, in the drug group before and after the intervention

Tests and parameters	Control (before) Mean ± SD	Control (after) Mean ± SD	*P* value (Before-after$_{Control}$)	Intervention (before) Mean ± SD	Intervention (after) Mean ± SD	*P* value (Before-after $_{Intervention}$)
TAC	3227.79 ± 192.53	3281 ± 279.37	0.82	3474 ± 257.48	3579.50 ± 210.16	0.75
TOS	74 ± 9.10	71.49 ± 8	0.83	113.28 ± 12.57	79.70 ± 8.25	0.02
MDA	11.84 ± 1.26	11.49 ± 6.23	0.78	10.14 ± 1.75	8.30 ± 1.24	0.42
catalase	4.87 ± 1.22	7.60 ± 1.38	0.14	4.44 ± 0.68	6.19 ± 0.94	0.10
paraoxonase	21.97 ± 1.61	20.75 ± 1.38	0.55	22.03 ± 1.43	22.61 ± 1.35	0.55
neopterin	2.02 ± 0.56	0.97 ± 0.14	0.06	1.81 ± 0.36	1.54 ± 0.27	0.13
NIHSS	**Control**		*P* value	**Intervention**		*P* value
NIHSS at the first visit	8.64 ± 1.1.19		0.14*	6.55 ± 1		–
NIHSS After 24 h	6.82 ± 1.24		0.134*	4.38 ± 0.99		< 0.001**
NIHSS After 2 weeks	5.93 ± 1.36		0.050*	2.94 ± 0.70		< 0.001**
NIHSS After 1 month	6 ± 1.63		0.02*	2.22 ± 0.56		0.001**
NIHSS After 3 months	3.53 ± .71		0.057*	1.83 ± 0.51		0.001**

**Calculated for each NIHSS after a certain period post-intervention when compared to NIHSS reported at the first visit (6.55 ± 1) in intervention group

*Calculated for each NIHSS after a certain period post-intervention when compared to NIHSS evaluated in corresponding control group

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at the first visit (6.55 ± 1), we noticed a notable difference for each studied period: NIHSS/24h$_{Intervention}$, P value < 0.001, NIHSS/2weeks$_{Intervention}$, P value < 0.001, NIHSS/1month$_{Intervention}$, P value $= 0.001$, NIHSS/3months$_{Intervention}$, P value $= 0.001$. (Table 2) The provided data showed a significant efficiency of NAC in reducing NIHSS in intervention group over the time.

To have a better understanding of the effects of NAC on test parameters of individuals placed in intervention group, we divided this group into two subgroups namely the Intervention-responsive group determined by the increased serum levels of TAC, paraoxonase, and catalase and also decreased serum levels of TOS, MDA, and neopterin after the intervention compared to the level measured before the intervention and the Intervention-non-responsive group, which included individuals in intervention group with decreased serum levels of TAC, paraoxonase, and catalase and increased serum levels of TOS, MDA, and neopterin after the intervention according to our data (Table 3).

It is clearly shown that there is a significant difference between each studied parameter between the two subgroups before and after the intervention. In the non-responsive group, administration of NAC decreased TAC ($P < 0.001$), increased TOS ($P < 0.001$), increased MDA ($P = 0.002$) but decreased paraoxonase ($p < 0.001$), catalase ($p = 0.03$), and neopterin ($p = 0.001$).

Additionally, in responsive group, administration of NAC increased TAC ($P < 0.001$), decreased TOS ($P < 0.001$), decreased MDA ($P < 0.001$), but increased

paraoxonase ($p < 0.001$), catalase ($P < 0.001$), and decreased neopterin ($p = 0.001$).

4 Discussion

In the present study, we used a panel of biomarkers and parameters to investigate the oxidative stress pre/post-intervention including MDA, neopterin, TOS, catalase, paraoxonase, and TAC. Oxidative stress promotes the production of ROS that react with other biomolecules among them lipids, to generate compounds including MDA. Therefore, measuring the plasma levels of MDA mirrors the levels of lipid peroxidation and severity of oxidative stress [29]. Neopterin, an inflammatory biomarker, is produced in macrophages after the induction of guanosine triphosphate (GTP) cyclohydrolase I by IFN-γ [30] (well-known as a proinflammatory cytokine [31, 32]). TOS evaluates the overall pro-oxidant state, whereas the total antioxidant status (TAS) reflects the overall antioxidant state. TOS/TAS ratio (oxidative stress index) is a beneficial parameter to study oxidative stress [33]. Catalase (CAT, EC.1.11.1.6) is a portion of the enzymatic antioxidant system that mediates the catalysis of H_2O_2 [34, 35]. The paraoxonase family of antioxidant enzymes includes paraoxonase 1 (PON1), PON2, and PON3. PON2 and PON3 act as intracellular enzymes to regulate the production of mitochondrial superoxide anions [36]. TAC is a strategy to monitor the free radical-antioxidant balance in tissues or body fluids [37].

Although our results showed no notable difference between intervention and the control groups, investigation of NAC effects between two subgroups of

Table 3 Comparison of mean serum levels of TAC, TOS, MDA, paraoxonase, catalase, and neopterin between intervention-responsive and non-responsive subgroups before and after the intervention

Variables	After intervention Mean ± SD Intervention-non-responsive group	Before intervention Mean ± SD Intervention-non-responsive group	*P* value
TAC	2791.15 ± 760.04 ($n = 20$)	3606 ± 1003.20 ($n = 20$)	< 0.001
TOS	102.04 ± 23.40 ($n = 15$)	65.54 ± 22.25 ($n = 15$)	< 0.001
MDA	13.60 ± 6.51 ($n = 11$)	8.45 ± 3.48 ($n = 11$)	0.002
paraoxonase	20.50 ± 4.71 ($n = 13$)	26.19 ± 4.77 ($n = 13$)	< 0.001
catalase	3.61 ± 2.81 ($n = 14$)	6.53 ± 5.38 ($n = 14$)	0.03
neopterin	1.74 ± 0.73 ($n = 10$)	1.07 ± 0.77 ($n = 10$)	0.001
Variables	**Intervention-responsive group**	**Intervention-responsive group**	**–**
TAC	4208.1 ± 767.15 ($n = 17$)	3072.5 ± 938.61 ($n = 17$)	< 0.001
TOS	58.13 ± 30.46 ($n = 22$)	115.55 ± 56.69 ($n = 22$)	< 0.001
MDA	8.18 ± 4.58 ($n = 26$)	11.89 ± 6.75 ($n = 26$)	< 0.001
Paraoxonase	22.68 ± 6.34 ($n = 24$)	19.73 ± 6.14 ($n = 24$)	< 0.001
Catalase	8.80 ± 4.97 ($n = 23$)	3.42 ± 2.44 ($n = 23$)	< 0.001
Neopterin	1.11 ± 1.05 ($n = 27$)	2.21 ± 2.17 ($n = 27$)	0.001

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intervention group provided data indicating a sharp and significant difference before and after NAC administration. One possible explanation is the low number of recruited individuals. Using higher allowed doses or multiple administration instead of a one-dose strategy may also be helpful to magnify the efficiency of NAC. The main limitation of our study was the small size of the studied groups. We could include 37 individuals in the intervention group and the same number in control group. Additionally, we did not measure the levels of ROS, however, we recommend measuring ROS levels pre/post-intervention to propose a possible mechanistic pathway of NAC effects on reducing oxidative stress. In this study, in the group of stroke individuals who responded to treatment, serum levels of TAC, paraoxonase, and catalase increased significantly, while serum levels of MDA and TOS decreased significantly. Oxidative stress has been reported as one of the mechanisms involved in ischemic brain injury [28]. Acute ischemia by a variety of mechanisms, including stimulation of *N*-methyl-d-aspartate (NMDA) receptors, mitochondrial dysfunction, activation of neuronal nitrite oxidase (NOS), induction of NOS by cyclooxygenase 2, autooxidation of catecholamines, and metabolism of free fatty acid such as arachidonic acid can increase the production of free radicals [38, 39]. Also, different studies have shown the effect of antioxidants on the treatment of stroke. Most of these studies have considered the role of antioxidant supplements in reducing cardiovascular disease [40, 41]. The increase in catalase activity after receiving NAC treatment is probably related to the ability of NAC to directly remove ROS [42]. The results of this study were consistent with the results of previous studies [43, 44], in which NAC supplementation was reported to improve learning and memory deficits in a rat model with hyperglycemia-induced oxidative stress. In the case group of the studied Wistar rats, lipid peroxidation in the brain stem and cerebral cortex increased, whereas the enzyme activity of superoxide dismutase, catalase, and glutathione reductase decreased. Interestingly, NAC supplementation improved memory deficits and significantly raised the catalase activity in the cerebral cortex (22.1%) and brain stem (17.6%) [43]. Lipids, especially unsaturated fatty acids in cell membranes, are among the most sensitive biological molecules that are exposed to ROS attack, which increases the rate of lipid peroxidation [45]. MDA is the main indicator of the oxidation of unsaturated fatty acids and is considered an indicator of oxidative stress in the pathogenesis of many diseases [46]. MDA was measured as a sensitive marker of lipid peroxidation in the past using TBA thiobarbituric acid and the colorimetric method. Since the sensitivity of the colorimetric method for measuring MDA is not high enough

due to the intervention of chromogens or dyes, the sensitive and specific HPLC method was used. The measurement of the MDA-TBA intermediate composition by HPLC is more specific than the colorimetric method. In the present study, a decrease in MDA levels was observed due to the administration of NAC, most probably due to the ability of NAC to directly remove free radicals and inhibit lipid peroxidation by ROS. Two studies had previously reported an increase in MDA in stroke and a decrease after NAC administration and their results were consistent with ours [47, 48]. Li et al., to have a better understanding of the effect of NAC on MDA in heat stress-induced intestinal injury, designed a study consisting of in vivo (NAC pretreated -C57BL/6 mice kept in a pre-warmed incubator until reaching a rectal core temperature of 42 °C) and in vitro (using IEC-6 cells incubated for 2 h at 43 °C to induce heat stress and pretreated with NAC) experiments [49]. Their results showed that in both study models, NAC treatment could reduce the heat stress-induced levels of MDA [49]. The present study showed that the serum level of paraoxonase-1 in the response group increased significantly compared to values measured before treatment, which was consistent with the findings of other studies [50, 51]. Paraoxonase-1 binds to HDL-C and removes the oxidized form of LDL-C in serum. Therefore, a change in lipid profile may affect serum paraoxonase activity. As a result, a decrease in serum paraoxonase can increase the oxidation of lipids [51]. Additionally, we showed that the serum levels of neopterin in the response group decreased significantly compared to pretreatment. Various studies have reported increased levels of neopterin in stroke and other diseases, which is consistent with our findings [52, 53]. But to the best of our knowledge, it has rarely been aimed to investigate the effect of NAC on neopterin levels. In the intervention-responding group, NAC significantly decreased serum TOS levels compared to pretreatment but increased serum TAC levels, which is consistent with the results of similar studies [54, 55]. These results may be due to NAC effects on reducing oxidative stress and thus reducing ROS. This study aimed to evaluate the effect of NAC on serum levels of oxidative inflammatory biomarkers in individuals with stroke. In confirmation of the results obtained from this study, we refer the readers to similar studies below. In a study conducted by Izadi et al. in 2012 on the role of NAC in reducing oxidative stress induced by diazinon in the liver and kidneys of rats, the results showed that administration of NAC reduces catalase activity. The results of this study were consistent with the present study [44]. In 2000, Cuzzocrea et al. investigated the effects of NAC on treatment of individuals with stroke, and the results showed that NAC reduced MDA as well as neopterin in individuals with

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cerebral ischemia. The results of this study are consistent with the present study [56]. Ozdemir et al. evaluated the effect of NAC and vitamin E on oxidative stress in children with major thalassemia and the results showed that serum TAC levels in individuals receiving NAC increased when compared to controls and serum TOS levels were decreased [55]. In 2020, Gerreth et al. conducted a study to comprehensively assess oral health status, salivary gland function, and oxidative stress in the saliva of individuals with acute stroke [54]. Their results showed that the level of TAC was not significantly different between the study group and the control group, while the level of TOS was significantly higher in stimulated and unstimulated saliva and stroke individuals. The results of this study are inconsistent with our results. This discrepancy can be due to the use of various drugs that may affect serum TAC and TOS levels [54]. Finally, in 2014, Shen et al. examined the effect of different levels of NAC diet on antioxidant factors and oxidative stress in Wistar rats and the results showed that in all groups receiving NAC with different doses, serum paraoxonase-1 level was increased. The obtained results are consistent with the results of the present study [51]. According to Table 2, a direct comparison between the measured parameters indicated no significant difference between the two studied groups, and however, NAC was found to act promising in reducing NIHSS and improving cognitive function in individuals with stroke. We propose that there could be other mechanisms of action through which NAC acts successfully to reduce NIHSS rather than our studied parameters mostly focusing on the antioxidant properties of NAC.

5 Conclusion

We found no notable difference in the results of TAC, TOS, MDA, neopterin, catalase, and paraoxonase in the control group before and after receiving standard treatment. Administration of NAC, although could improve antioxidant parameters in the intervention group before and after receiving NAC, however, the results were not statistically significant except for TOS. Additionally, the results for TOS between intervention and control groups were significantly different ($P = 0.02$). Then, we studied the results of the test panel after dividing the intervention group into responsive and non-responsive groups and reported a significant decrease in serum levels of oxidative inflammatory biomarkers in the intervention-responsive subgroup compared to levels measured before treatment. The results showed that administration of NAC in this subgroup could increase TAC ($P < 0.001$), paraoxonase ($p < 0.001$), and catalase ($P < 0.001$) and could decrease TOS ($P < 0.001$), MDA ($P < 0.001$), and neopterin ($p = 0.001$). It can be concluded that NAC can be used as a complementary drug and a powerful antioxidant in reducing oxidative stress and improving cognitive function in individuals with stroke.

Abbreviations:

ROS	Reactive oxygen species
MDA	Malondialdehyde
NAC	*N*-Acetylcysteine
NIHSS	National Institutes of Health Stroke Scale
TAC	Total antioxidant capacity
TOS	Total oxidative status
GSH	Glutathione
TBA	2-Thiobarbituric acid
MRI	Magnetic resonance imaging

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The protocols of this study are approved by the ethics committee of Kermanshah University of Medical Sciences (ethics code IR.KUMS.REC.1399.563).

Consent for publication
Patients signed informed consent regarding publishing their data and photographs.

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

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