




## GFAP and Neuron Specific Enolase (NSE) in the Serum of Suicide Attempters

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Received: 15 Nov 2021

Published: 7 Sep 2022

### Abstract

**Background:** To determine whether neuronal damage and/or neuroinflammation exist in the brain of suicide attempters and to find a novel biological biomarker to help distinguishing high risk individuals with suicide behavior, we aimed to measure glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), and nerve growth factor (NGF) in suicide attempters.

**Methods:** In the present case-control study, the serum level of NSE, GFAP, and NGF were measured quantitatively in 43 suicide attempters and 43 healthy control participants aged 18 to 35 years. Data were analyzed using the nonpaired t test followed by the Mann-Whitney posttest.

**Results:** The mean serum level of NSE and GFAP were significantly higher in suicide attempters compared with healthy control individuals ( $p = 0.003$ ,  $p = 0.001$ , respectively), while no significant difference was detected in NGF serum level between the 2 groups.

**Conclusion:** Our findings of increased level of NSE along with the significant increase in GFAP would propose the presence of low grade neuroinflammation in the brain of these participants. NSE/GFAP might be good markers that is easily accessible and can be considered as prognostic markers in high-risk suicide attempters.

**Keywords:** GFAP, NGF, NSE, Neuronal Inflammation, Suicide Risk

**Conflicts of Interest:** None declared

**Funding:** Ilam University of Medical Sciences (grant number: IR.MEDILAM.REC.1395.50- IR.MEDILAM.REC.1395.51)

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**Cite this article as:** Bagheri M, Ghaneialvar H, Oshnokhah M, Salari S. GFAP and Neuron Specific Enolase (NSE) in the Serum of Suicide Attempters. *Med J Islam Repub Iran.* 2022 (7 Sep);36:103. <https://doi.org/10.47176/mjiri.36.103>

### Introduction

Suicide behavior is a public health problem in societies and a leading cause of approximately 800,000 death worldwide (1). Various risk factors were reported to be implicated in suicide behavior (2), including psychosocial, environmental, familial, and genetic factors (3, 4). However, there are clear biological/ neurobiological changes that occur at a neurochemical level in suicide attempters (5).

Finding specific biomarkers has been attracted much interest of scientists worldwide. Neuron specific enolase (NSE), which is measurable in blood and cerebrospinal

fluid (CSF), can be potentially a valuable biomarker in evaluating neuronal damage, brain injury, and inflammation (6-9). Enolase 2 is measurable in blood and CSF and known as gamma enolase or NSE. It is a cytoplasmic enzyme and works in the glycolytic pathway (10, 11). Two different isoenzymes of NSE presents in CNS;  $\gamma\gamma$  is limited to neurons, while  $\alpha\gamma$  is expressed in glia cells (12, 13). NSE is associated with different clinical conditions, ischemia, hypoxia, metabolic disease, inflammatory, and neurodegenerative disease (14, 15). Loss of neurons and synaptic

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#### ↑What is “already known” in this topic:

Various studies show that suicide behavior is a complex and multifactorial phenomenon. Different cerebrospinal fluid /blood/ brain tissue markers may be involved in human behavior.

#### →What this article adds:

Here, increased serum level of glial fibrillary acidic protein and neuron specific enolase were shown in suicide attempters regardless of history of major depressive disorders. This may amplify the importance of biological factors at higher steps in suicide behavior.

connections are the most common features of neurodegenerative disease, and more increase in NSE level in serum and CSF implies the more neuronal loss (6). Furthermore, NSE disturbances in neuroinflammation is mediated by reactive microglia and astrocytes. Consequently, NSE level in the serum can be considered as a reliable biomarker with diagnostic and prognostic potential for neuronal damage and/or neuroinflammation in different diseased conditions.

On the other hand, astrocytes as special types of glial cells play important roles in development, synaptic functions, brain metabolism, and homeostasis of fluids, ions, pH, and neurotransmitters in healthy brains (16). Furthermore, astrocytes go through various cellular and functional remodeling in response to brain injury, infection, inflammation, and neurodegeneration, which is reflected through changes in the cellular upregulation of glial fibrillary acidic protein (GFAP). GFAP as an intermediate filament protein is expressed primarily in the brain astrocytes and interestingly penetrates into the cerebrospinal fluid, which can be detected and measured in blood stream. Also, there are much evidence regarding the GFAP measurement in blood/sera (17, 18).

There is evidence that the density of GFAP-immunoreactive astrocytes is decreased in the left hippocampi in major depressive disorder (19). In addition, GFAP is differentially expressed in cortical and subcortical regions in depressed suicide attempters (20). In 2015, Nagy et al found significant abnormalities in astrocytes along with DNA methylation patterns in depression and suicide behavior (21).

Furthermore, NGF as a neuropeptide is firstly introduced in 1956 and referred to neurotrophin family besides brain-derived neurotrophic factor (BDNF) and neurotrophins 3, 4/5, and 6. NGF can give rise to neurotrophic, metabotropic, and immunotropic effects through many physiological mechanisms. Fluctuation in NGF serum level has been found in neurodegenerative diseases (e.g., AD and PD), psychiatric disorders (e.g., depression and schizophrenia), as well as non-neuronal disorders (diabetes mellitus and obesity) (22).

Consequently, the aim of this study was to measure the serum levels of NSE and GFAP in suicide attempters and healthy controls to find a valuable marker in suicide behavior.

## Methods

### Participants

The current case-control study consists of 2 different groups: suicide attempters and healthy controls (non-suicidal). The former included 43 hospitalized suicide attempters who were admitted to the emergency room after a suicide attempt during in the spring of 2018 and the summer of 2019, and the latter included healthy controls without a history of suicide. We recruited participants with no history of suicide in the same society for the control group at the same time with the suicide attempters' sample collection through announcement. The both included equal number of male and female participants. We did not ask for the number of attempts in the attempter's group, as it was not noticed in the inclusion /exclusion criteria for the experiment. In the primary study, 230 participants were included;

however, after applying the inclusion and exclusion criteria, a total of 86 participants were selected. All participants were matched by sex and age; they were aged 18 to 35 years and interviewed by a trained nurse using a prepared structural questioner for demographic, psychiatric, and medical history. They were all medication free for at least 8 weeks. Notably, suicide attempters and controls were evaluated for the major depressive disorder (MDD) by Beck the Depression Inventory (BDI-II), and participants without MDD were selected for the experiment (the related data are not presented here).

The study protocol was approved by the Ethics Committee of Ilam University of Medical Sciences, Ilam, Iran (IR.MEDILAM.REC.1395.50 and IR.MEDILAM.REC.1395.51).

Written informed consent was obtained from all participants or their families.

### Blood Sample Collection

All participants were fasting (at least from midnight) before collecting the blood sample. Approximately 10 mL of blood samples were drawn in all individuals (suicide attempters and normal controls) from the participants' antecubital vein between 8:30 AM to 10:30 AM. In suicide attempters, samples were taken following an overnight admission after the failed attempt. In the controls, all samples were collected at the same time (8:30 to 10:30) in fasting status as well. Afterward, the whole blood was collected in vacutainer tubes containing no anticoagulant, allowing it to clot at room temperature for almost 30 minutes, and centrifuged at 1000 x g for 15 minutes (no later than 60 min after sample collection). The supernatant was aspirated carefully as serum, inspected precisely for turbidity before storing at -80 °C.

### Inclusion & Exclusion Criteria

**Inclusion criteria:** Being a male or female participant aged 18 to 35 years, admitted to the emergency ward after a suicide attempt, normal controls without a suicide attempt, and being medication free for at least 8 weeks were the inclusion criteria for this study.

**Exclusion Criteria:** A history of metabolic syndrome; brain injury during/after suicide attempt; use of a violent method, including a knife, hanging, shooting, falling, and et cetera, which could cause head trauma and injury during the attempts; and medication use during the last 8 weeks.

Notably, the means of suicide for attempters was nonviolent, including acetaminophen, tramadol, and oil drinking. Suicide with violent methods such as self-immolation, hanging, and shooting with firearms were excluded. Suicide attempt was not severe in all attempters. They did not experience brain trauma, brain damage, or hypoxia during or after the attempt.

### Laboratory Analysis

#### NSE Measurement

This assay employed the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique. To measure human NSE, the manufacturer's instructions (Quantikine Human Enolase 2 Immunoassay, R&D system, Inc)

was used. All assays were performed in duplicate. In brief, a monoclonal antibody specific for human enolase 2 had been precoated onto a 96 wells microplate. Assay diluent was pipetted to each well of microplate. Next, 50  $\mu$ L of standard/control/ sample was added per well and sealed and incubated for 2 hours at room temperature on a horizontal microplate shaker. Following the proper washing, human enolase 2 conjugate was added to each well, sealed again, and incubated for 2 hours at room temperature while shaking. Substrate solution was added after washing and incubated for 30 minutes in darkness at room temperature. Then, stop solution was used, the color in the wells changed from blue to yellow. The density of color in each well determined at 450 nm within 30 minutes. To correct the optical imperfection, the readings at 450 nm were subtracted from readings at 570 nm. Serum NSE concentration was expressed as ng/mL.

#### GFAP Measurement

The Human GFAP ELISA kit (OKEH00110-Lot#KC1310; Aviva System biology) was used for the quantitative measurement of a target protein in human sera was based on the ELISA technology. The specific capture antibody for GFAP was coated on a 96 well microplate. Titrated standards and diluted serum samples or blank were pipetted into the wells of GFAP microplates, sealed carefully, and incubated at 37 °C for 2 hours. Then, the biotinylated GFAP detector antibody was added to the wells and incubated again at 37 °C for an hour. Avidin-HRP conjugate was added after washing and incubated at 37 °C for 60 minutes. Finally, stop solution was used for changing color to yellow immediately. The OD absorbance was read within 5 minutes at 450 nm. The serum GFAP level was expressed as ng/mL.

#### NGF Measurement

The human beta-NGF ELISA kit (RAB0380, Sigma-Aldrich) was used for the quantitative measurement of a target protein in human sera. Standards and the serum were pipetted into the wells, and incubated overnight at 4 °C with gentle shaking. Following washing, the biotinylated detection antibody (1:80), specific for the human NGF, was incubated for an hour at room temperature with gentle shaking. After washing, HRP-streptavidin solution (1: 800) was added and remained for 45 minutes at room temperature; gentle shaking was necessary. Next, each well was incubated with the ELISA colorimetric TMB reagent for 30 minutes in darkness. Color develops in proportion to the amount of NGF bounding. Finally, the density of the color was measured at 450 nm immediately. The serum NGF level was expressed as pg/mL.

#### Statistical Analysis

Data were analyzed using the nonpaired t test followed by the Mann-Whitney post-test. Graph Pad Prism VI (GraphPad software Inc) was used for the analysis and results are presented as mean  $\pm$  SD;  $P < 0.05$  was considered statistically significant. The Shapiro-Wilk test was used for normality test. The sample size was calculated using the

formula for mean comparison between the 2 independent groups.

#### Results

A total of 86 individuals were included in this study. The suicide attempted group ( $n = 43$ ) had at least 1 episode of suicide attempt, and the control group ( $n = 43$ ) had no history of suicide attempt.

Based on the demographic questioner, all participants were medication-free for at least 8 weeks before the suicide attempt. The suicide attempters used nonviolent methods. In addition, the age of participants in the both groups were between 18 to 35 years; suicide attempters (mean age,  $27.3 \pm 0.86$  years) and the non-suicidal participants (mean age,  $28.09 \pm 0.75$  years) (Table 1). The mean of body mass index (BMI) in suicide attempters and non-suicidal participants was  $24.7 \pm 0.5$  and  $25 \pm 0.3$  kg/m<sup>2</sup>, respectively, which did not differ significantly (Table 1).

The mean concentration of serum NSE in the suicide group was  $3.88 \pm 0.26$  ng/mL, which was significantly higher compared with the controls ( $2.86 \pm 0.25$  ng/mL;  $p = 0.003$ ) (Fig. 1).

NSE levels were increased significantly in the suicide attempter group ( $3.88 \pm 0.26$  ng/mL) when compared with the controls ( $2.86 \pm 0.25$  ng/mL). The bars represent SEM ( $n = 43$  in each group;  $p = 0.003$ ).

In addition, the mean concentration of GFAP in the serum of suicide attempters was  $0.25 \pm 0.003$  ng/mL, while it was  $0.24 \pm 0.001$  ng/mL in the healthy controls ( $p = 0.001$ ) (Fig. 2).

Moreover, the mean concentration of NGF in the serum of suicide attempters was  $45.7 \pm 1.2$  pg/mL, while it was  $44.4 \pm 1.5$  pg/mL in the healthy controls; there was no significant difference between the 2 groups (Fig. 3).

#### Discussion

Using human serum specific antibodies and enzyme linked-immunosorbent assay, we aimed to measure serum NSE, NGF, and GFAP levels in suicide attempters to find whether these factors can be considered as potential biomarkers in the blood stream. Notably, for the first time, we demonstrate that serum NSE level along with the serum GFAP level were significantly higher in suicide attempters.

NSE can play dual roles in neuroinflammation and neuroprotection via different signaling pathways (6). Recent studies on NSE confirmed that it could be a potential biomarker for assessing neuronal damage, prognosis of brain injury, and neurobehavioral outcome in neurologic conditions (23, 24). Haque et al in 2018 declared that the neuronal damage directly correlates with an increased level of NSE in the serum and CSF of patients (6). In contrast with Wiener and his colleagues who showed no significant

Table 1. Age and Body Mass Index

| Participant Characteristics | Suicide Attempters | Healthy Controls |
|-----------------------------|--------------------|------------------|
| Mean age                    | $27.3 \pm 0.86$    | $28.09 \pm 0.75$ |
| Mean BMI                    | $24.7 \pm 0.5$     | $25 \pm 0.3$     |
| Total n                     | 43                 | 43               |

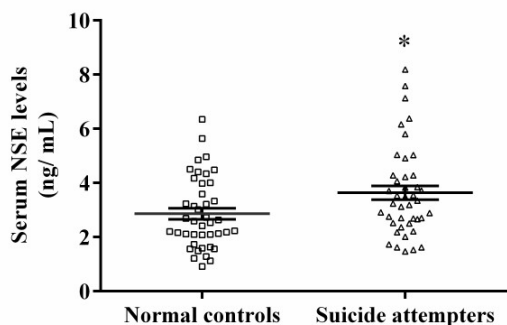


Fig. 1. NSE serum levels in suicide attempters and healthy controls

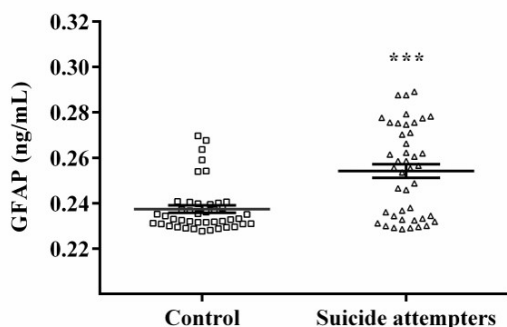


Fig. 2. GFAP serum levels in suicide attempters and healthy controls. GFAP levels were increased significantly in the suicide attempter group ( $0.25 \pm 0.003$  ng/mL) when compared with the controls ( $0.24 \pm 0.001$  ng/mL;  $P = 0.001$ ).

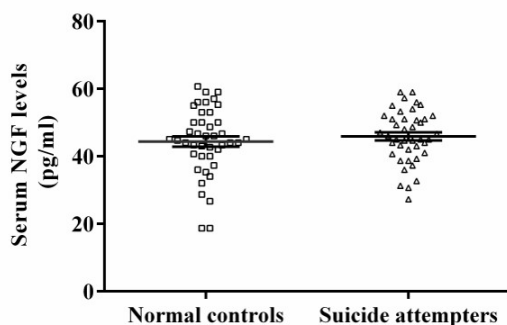


Fig. 3. NGF serum levels in suicide attempters and healthy controls. NGF levels were increased slightly but not significantly in the suicide attempter group ( $45.7 \pm 1.2$  pg/mL) when compared with the controls ( $44.4 \pm 1.5$  pg/mL). The bars represent SEM ( $n = 43$  in each group).

changes in NSE levels along with the severity of depression and suicidal ideation in drug-naïve young adults, our findings showed an increased level of NSE in suicide attempters (25). Although there are not much studies about NSE on suicide attempters, a recent study showed a significant increase of NSE and S100B in MDD patients irrespective of suicide, which confirms the association of NSE and behavior (26). An increase in serum NSE in the present study was not related to brain trauma/damage, as we used nonviolent method; however, this might reflect neuronal distress in suicide attempters.

GFAP is expressed in astrocytes and widely involved in the regulation of synaptic transmission and immune

reactions (21, 27). Axel petzold in 2015 introduced GFAP as a body fluid biomarker for glial pathology in different disease conditions (28). Here, we showed the elevated level of GFAP in the serum of suicide attempters. GFAP elevation is associated with neuroinflammation (29), BBB disruption, and brain vascular inflammation (20, 30). Furthermore, there is much evidence suggesting astrocytic abnormality in depression and suicide behavior (20). In contrast with our findings, Nagy C. et al exhibited the downregulation of GFAP in prefrontal cortex tissue in patients with depression or those with completed suicide (21). Fluctuation in the GFAP level correlates with the severity of depression and suicide. On the other hand, GFAP expression differs through different brain regions and other parts of the brain, or CSF/bio fluid might show increased levels of GFAP if investigated (31).

Notably, all participants were between the ages of 18 and 35 and had no head injuries during the suicide; therefore, neither neurodegeneration nor brain injury is responsible for increased NSE, while they may indicate neuroinflammation in association with increased GFAP.

Finally, we measured NGF as one of the main members of the neurotrophins family. NGF is broadly found in cortex, hippocampus, and hypothalamus (32, 33) and plays a role in neuronal survival, differentiation, connectivity, and plasticity (32, 34). Although we did not observe any significant fluctuations in NGF levels, some have found reduced NGF levels in MDD and suicide victims (34). In addition, several studies have suggested that neurons are involved in the pathophysiology of MDD and other psychiatric disorders (32). The serum NGF level was unchanged in this study possibly due to the exclusion of major depression attempters from the study.

**Conclusion**

In summary, we reported increased levels of GFAP and NSE in the sera of suicide attempters, which means neuroinflammation in the brains of these participants. Elevated NSE levels as well as elevated GFAP levels may be appropriate markers available in high-risk patients. However, further investigations with larger samples and different suicide attempt/completion groups are needed to find the best verdicts.

**Acknowledgment**

This study was supported by a grant from Ilam University of Health Sciences. The authors thank Mr. Shaabani from the emergency department of Shahid Mostafa Khomeini Hospital, Ilam, Iran for his assistance in the laboratory.

**Abbreviation**

- NSE: neuron specific enolase
- GFAP: glial fibrillary acidic protein
- NGF: nerve growth factor
- MDD: major depressive disorders

**Ethics approval**

The current manuscript has been approved in the ethic committee of Ilam University of Medical Sciences, numbered IR.MEDILAM.REC.1395.50 and IR.MEDILAM.REC.1395.51.

**Conflict of Interests**

The authors declare that they have no competing interests.

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