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Original article

Associations between glutamic acid decarboxylase antibodies, oxidative stress markers, and cognitive capacity in adolescents who stutter

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ABSTRACT

In this study, we amid to evaluate the correlation between the change in the expressed levels of anti-GAD antibodies titers, oxidative stress markers, cytokines markers, and cognitive capacity in adolescents with mild stuttering. Eighty participants (60 male/20 female) with the age range of 10–18 years with moderate stutteringparticipated in this study. To assess the stuttering and cognitive function, stutteringseverity instrument (SSI-4; 4th edit.)and the LOTCA-7 scores assessment were applied respectively in all subjects. In addition, serum GAD antibodies, cytokines like TNF-α, CRP,and IL-6 withtotal antioxidant capacity and nitric oxide as oxidative stress markers were estimated using calorimetry and immunoassay techniques. The results showed that good cognitive capacity was reported in about 56.25 % of the study population (n = 45) with a 117.52 ± 6.3 mean LOTCA-7 score. However, abnormal cognitive function was identified in 43.75 % of the study population (n = 35); they were categorized into moderate (score 62–92, n = 35), and poor (score 31–62; n = 10). There were significant associations between cognitive capacity reported and all biomarkers. The expression of GAD antibodies is significantly associated with the degree of cognitive capacity among students with stuttering. Significant association with the reduction (P = 0.01) in LOTCA-7 score domains, particularly orientation, thinking operations, attention, and concentration among students with variable cognitive capacity compared to controls. In addition, the expressed higher GAD antibodies in students with moderate and poor cognitive capacity showed to be significantly correlated with both elevated concentrations of cytokines; TNF-α, CRP, and IL-6, and the reduction of TAC and nitric oxide (NO) respectively. This study concludes that abnormality of cognitive capacity showed to be associated with higher expression of GAD antibodies, cytokines, and oxidative stress in school students with moderate stuttering.

1. Introduction

Stuttering is diagnosed in subjects with disfluent patterns and involuntary disruptions in speech with irregular timing of phonemes. In addition, subjects with stuttering are suffering from delaying or inhibition in the capacity to communicate efficiently with others (Bloodstein and Bernstein-Ratner, 2008). Subjects with stuttering showed also some difficulties in repetitions of words or syllables with frequent hesitations or pauses (Di Simoni, 1974; Bloodstein, 1960). Around 1.4 % of children and adolescents worldwide are suffering from stuttering (Craig et al., 2002). Whoever, around 8 % of adults have stutter with ~1% prevalence as well (Yairi and Ambrose, 2013; Stuttering Foundation of America (SFA), 2017).

Biologically, subtle differences in brain function and anatomy were identified in subjects with stuttering. The changes lead to a neurodevelopmental disorder affecting speech-motor control (Neef et al., 2015). In addition, most medical studies showed that cellular physiological, genetic, neurological, and psychological disorders were the most causes of stuttering which was significantly affected by environmental factors (Yaruss and Quesal, 2004; Guitar, 2013; Howell, 2007). Genetic factors significantly affect in

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71 % of stuttering, followed by a ratio of 29 % of stuttering results from environmental factors (Peters and Guitar, 1991). Thus, behavioral, cognitive components, psychological, and social factors are significantly associated with the progression of stuttering (Yairi et al., 1996; Månsson, 2000).

In addition, some other risk factors such as cognitive, and specific emotions like fear, anxiety, embarrassment, and irritation were significantly diagnosed in subjects with stuttering (American Psychiatric Association (APA), 2012; Yaruss and Quesal, 2004). More cognitive parameters like "negative speech-related attitudes, difficulties in communicating with others, and perfectionistic thinking showed to be effective on the status of stuttering" (Amster and Klein, 2004; Boey, 2008; Guitar, 2013).This in turn leads to avoiding behavior and hide from possible negative reactions (Amster and Klein, 2004; Boey, 2008; Guitar, 2013).In-person who stutters, the difficulties and biological variability in cognitive abilities and their association with impacton speech performance among stuttering subjects will be a valuable means to the underlying deficitswhich help in diagnosis as well as therapy trials (Amster and Klein, 2004; Boey, 2008; Guitar, 2013; Weston and Riolo, 2007).

Previous studies showed that "complex cognitive and social growth were significantly associated with different physiological and physical in maturation period of adolescence as the transition sign from childhood to adulthood" (Yairi et al., 1996.Predictive; Spear, 2000; Kelly, 2007; Lincoln et al., 2006). Thus, it was reported previously that the existence of stuttering is considered an extra main factor inincreasing both "the level of stress andnegative psychological states" (Spear, 2000; Kelly, 2007; Lincoln et al., 2006). Thus,"more severe negative psychological states like anxiety, the decline in the scores of mental health, and; depression was recognized in association with stuttering in adolescents" (Yairi et al., 1996.Predictive; Spear, 2000; Kelly, 2007; Lincoln et al., 2006; Bricker-Katz et al., 2009).

Cognitive decline as a whole showed to be associated with a change in various biological and physiological factors (Stine-Morrow et al., 2008; Atti et al., 2010; Whitson et al., 2010). In addition, various cellular disorders like increased oxidative stress and free radical damage were reported in association with a cognitive impairment which significantly increased the distortion of brain function and anatomy (Song et al., 2009; Lovell and Markesbery, 2007; Butterfield and Sultana, 2007), whereas "the impairment in brain function pathologically accompanied by tissue injury, neural cell loss, and a disorder in the expression of neural and enzymatic biomarkerssuch as glutamic acid decarboxylase (GAD)" (Garde et al., 2000; De Groot et al., 2002; Cobb et al., 1995). It was reported that "the changes in modulation as well as neural network activity significantly associated in such away with diminishing in the synthesis of γ-aminobutyric acid (GABA)" [34–3].

Previous studies showed that the GABAergic system as a marker of neurological disorders is significantly affected by a decline in cognitive function. Cognitive problems"significantly effect on GABAergic system, leading to the production of glutamic acid decarboxylase antibodies (GADAs)" (Saiz et al., 2008; Ali et al., 2011).

In numerous neurobiological activities, the production of c-aminobutyric acid (GABA) as an essential neurotransmitter in several biological activities was controlled by the expression of two key enzymes (GAD65, GAD67) (Tinsley et al., 1997; Saidha et al., 2010; Black et al., 1998; Pinal and Tobin, 1998)."The regulation of synapse formation between neurons was linked with an increase in the enzymatic activity of GAD65, rather than GAD67, which could help in diagnosing of more brain dysfunctions" (Pinal and Tobin, 1998; Saiz et al., 2008; Tohid, 2016). GAD65Ab showed to be linked with more neurological diseases like epilepsy, stiff person syndrome, and autoimmune encephalitis. As an early manifestation, these diseases collectively may cause cognitive dysfunction (Tohid, 2016).

In addition, "recent and previous research works reported a significant association between cellular oxidative stress measured by nitric oxide (NO), lipid peroxide malondialdehyde (MDA), imbalance in antioxidant status measured by cellular enzymes superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity, and different inflammatory cytokines in subjects with cognitive impairments at different ages" (Li et al., 2016; Alghadir et al., 2021; Al-Rawaf et al., 2021; Kandlur et al., 2020; Padurariu et al., 2010; Baierle et al., 2015; Alghadir et al., 2021).

No or few studies explored the biological influence of glutamic acid decarboxylase (GAD) and oxidative stress markers on cognitive function among adolescents with mild stuttering.So, we hypothesized in our study that the biological changes in GAD expression levels with the disorder in the oxidative stress and inflammatory cytokines profiles in the serum of adolescents with mild stuttering greatly affected cognitive function.

In this regard, in our study, we triedto identify the potential correlation between the expression of anti-GAD antibodies titers, oxidative stress markers, cytokines markers, and cognitive capacity in adolescents with mild stuttering.

2. Materials and methods

2.1. Participants

One hundred school students with the same academic curricula were randomly recruited in this study. Only, 80 students (60 male/20 female) with the age range of 10–18 years diagnosed as moderate stuttering were suitable to participate as per the inclusion criteria. "Based on the use of one standardized questionnaire (DASS-42; score 15–18) and one standardized test material (SSI-4; a score of 18–24; percentile rank, 12–40),the students diagnosedfor mild stutteringandstress as previously reported" (Simen et al., 2011; Brian et al., 2011).Students with several "endocrine, immune, psychiatric, eating disorders, or received glucocorticoids or anti-inflammatory drugs that may interfere with cytokine and oxidative blood profiling or cognitive ability measurements were excluded from this study" (Simen et al., 2011; Brian et al., 2011). In addition, "students were excluded if they had any chronic diseases, abnormal iron status, anima, or mental diseases that could prevent them from participating in this study" (Simen et al., 2011; Brian et al., 2011).The procedures of the research were reported in Fig. 1. The study protocol was reviewed and approved by the ethical committee of the Scientific Research, King Khalid Universityhas reviewed, discussed, and has been approvedbased up on the ethical guidelines of the 1975 Declaration of Helsinki in 28/01/2022, under file number ((ECM#2022–2128)—(HAPO-06 -B-001)). All subjects or their parents completed and signed informed consent.The demographic and baseline data ofstudents were shown in Table 1.

2.2. Assessment of stuttering severity

"Stuttering Severity Instrument Measurements: The Stuttering Severity Instrument (SSI-4), the 4th Edition, quantifies disfluency duration, frequency, and physical features in preschool children through adults" (Simen et al., 2011; Brian et al., 2011). "The SSI-4 enables the assessment of behavioral severity levels in readers and non-readers. Classification of stuttering severity based on the total score and percentile ranks is as follows: no (total score, 10–17; percentile rank, 1–11), mild (total score, 18–24; percentile rank, 12–40), moderate (total score, 25–31; percentile rank, 41–77), severe (total score, 32–36; percentile rank, 78–95), and very

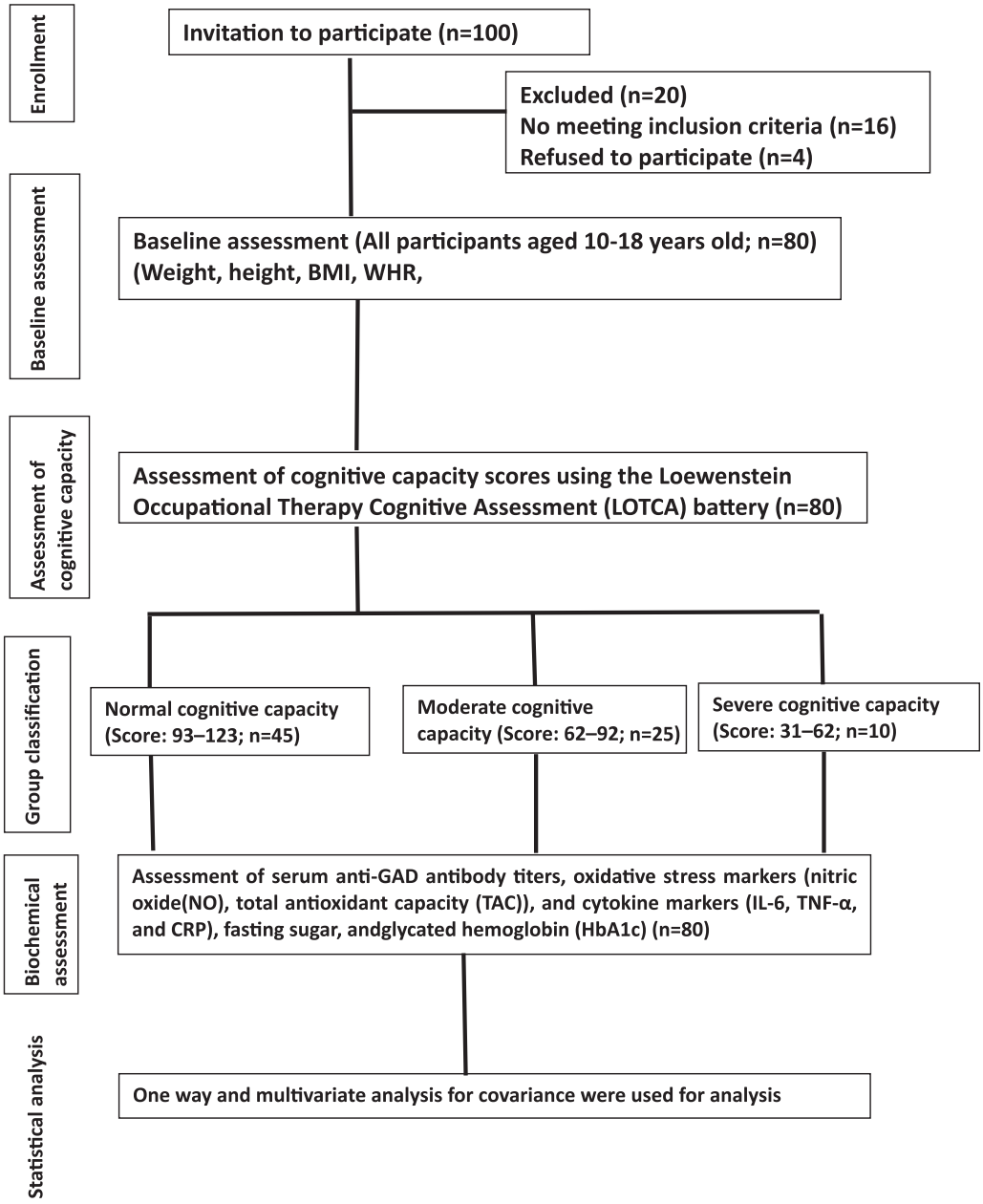

Fig. 1. Outline of study procedures.

severe (total score, 37–46; percentile rank, 96–99)" (Simen et al., 2011; Brian et al., 2011).The students of our study were diagnosed as mild stuttering with a total score of (18–24; percentile rank, 12–40).

2.3. Assessment of cognitive capacity

In this test, "the cognitive capacity of all students was assessed by using the Loewenstein Occupational Therapy Cognitive Assessment (LOTCA) score as previously reported" (Riley and Bakker, 2009; Almomani et al., 2014; Katz et al., 1989). "Seven major sections of the LOTCA test like orientation, visual perception, spatial perception, motor praxis, vasomotor organization, thinking operations, attention, and concentration were identified and divided into 26 subsections" (Riley and Bakker, 2009; Almomani et al., 2014; Katz et al., 1989). Moreover, "each subsection was scored respectively on a 4-point Likert scale" (Riley and Bakker, 2009; Almomani et al., 2014; Katz et al., 1989). In addition, "a combined score of each major section was "computed by adding the raw scores of each related subsection and the total LOTCA score was calculated the total scores of all subsections" (Riley and Bakker, 2009; Almomani et al., 2014; Katz et al., 1989).In Western and Arab populations, "the test of LOTCA score was previously evaluated and validated and the total LOTCA score ranged between 27 and 123, whereas, a higher score represents better cognitive function" (Riley and Bakker, 2009; Almomani et al., 2014; Katz et al., 1989; Josman et al., 2011). In this study, "the students with mild stuttering were divided into three groups based on their LOTCA test scores: good (93–123), moderate (62–92), and poor (31–61)" (Riley and Bakker, 2009; Almomani et al., 2014; Katz et al., 1989; Josman et al., 2011).

2.4. Blood analysis

Fasting "blood samples were collected early morning between 8 and 10 a.m from all students, centrifuged and the samples preserved in –20 ° until reused for analysis of subjected biochemical parameters" (Alghadir et al., 2016; Alghadir et al., 2015; Gorska-Ciebiada et al., 2015; Cortas and Wakid, 1990) as follows;

2.4.1. Analysis of blood sugar, glycated hemoglobin (HbA1c)

In all subjects, "a glucose oxidase and peroxidase (GOD-POD) colorimetric method (QuantiChrom Glucose Assay Kit, DIGL-100, BioAssay Systems, Hayward, CA, USA) were used to identify blood glucose. Also, a commercial kit (Bio-Rad, Richmond, CA, USA) was used to identify HbA1c" (Alghadir et al., 2016). In addition, "an ELISA (human insulin ELISA kit, KAQ1251, Invitrogen Corporation, Camarillo, CA, USA) were used to estimate the serum insulin level. The assays were performed according to the instructions provided by the manufacturers" (Alghadir et al., 2016).

2.4.2. Assessment of anti-GADAs antibody

"A colorimetric immunoassay ELISA technique using available a commercially available ELISA kit (RSR cat GDE96, RSR Limited, Cardiff, UK) was performed to identify serum anti-GAD65 antibody titers in all subjects" (Alghadir et al., 2015).GAD65 Abs are the most available and simply indicated as serum anti-GAD antibodies. Thus, "evaluating GAD65 antibodies by ELISA, provides GAD65 as a specific and sensitive potential marker of cognitive performance among school students with mild stuttering" (Alghadir et al., 2015).

2.4.3. Assessment of inflammation biomarkers

"A Quantikine Human Immunoassay ELISA kit (R & D System, Minneapolis, USA) was used to estimate the serum levels of CRP, IL-6, and TNF-α in serum samples obtained from all subjects" (Alghadir et al., 2015). "The data were interpreted according to the manufacturer's instructions. The minimum detectable concentrations were 1 × 10–5 mg/L for CRP, 0.7 pg/ml for IL-6, and 1.6 pg/ml for TNF-α" (Alghadir et al., 2015).

2.4.4. Assessment of oxidative stress parameters

2.4.4.1. Measurement of nitric oxide (NO). "Serum nitric oxide (NO) levels were measured as mentioned previously" (Alghadir et al., 2015; Gorska-Ciebiada et al., 2015; Cortas and Wakid, 1990). The content of NO"is measured as nitrate and nitrite residues using Griess reagent, which gives a purple azo-compound when reacted with nitrite" (Alghadir et al., 2015; Gorska-Ciebiada et al., 2015; Cortas and Wakid, 1990). "The produced azo-compound was measured at 450 nm against a standard of sodium nitrate, and the results were stated in mmol/l" (Alghadir et al., 2015; Gorska-Ciebiada et al., 2015; Cortas and Wakid, 1990).

2.4.4.2. Measurement of total antioxidant capacity (TAC). "Serum total antioxidant capacity (TAC) was measured using Colorimetric Assay Kit (Catalog #K274-100; BioVision Incorporated; CA 95,035 USA)" (Li et al., 2016). "The antioxidant equivalent concentrations were measured at 570 nm as a function of Trolox concentration according to the manufacturer's instructions" (Li et al., 2016).

"{Sa/Sv = nmol/µl or mMTrolox equivalent}" (Li et al., 2016).

"where: Sa is the sample amount (in nmol) read from the standard curve and Sv is the undiluted sample volume added to the wells. A higher value of TAC suggests a better health condition" (Li et al., 2016).

2.5. Sample Calculations

A sample comprising 80 school students with moderate stuttering was included in this study. Based on the scores of the LOTCA test analysis, the participants were then classified into three groups: good (n = 45), moderate (n = 25), and poor (n = 10). As previously reported, "the G ∗ Power program for Windows (version 3.1.9.7) was used to measure the power of the sample size of 80 subjects. Using the T-test and one tail with a significance level of 0.05, the total sample of 80 achieves a power of 95 % with an effect size of 0.35, Df = 78, critical t = 1.66, and no centrality -α = 3.32" (Moshage et al., 1995).

2.6. Statistical analysis

Statistical analysis was carried out with "SPSS (Statistical Package for Social Science) program version 17 for Windows (spssInc., Chicago, IL, USA). All data tabulated as mean ± SD" (Li et al., 2016; Alghadir et al., 2021). "The multivariate analysis of variance was used to compare the testing variables. Between subjects' effect of independent variables (i.e cognitive capacity) on biomarkers (e.g., serum GADantibodies, IL-6, TNF-α, CRP, TAC, and NO level) were investigated using analysis of covariance (ANCOVA), with age, BMI, and WHR, considered as covariates.Pearson's correlation coefficient was performed to study the correlation between the studied parameters. Multiple comparisons were rectified by using the Bonferroni correction. P-values<0.05 were considered statistically significant" (Li et al., 2016; Alghadir et al., 2021).

3. Results

In the current study, cognitive capacity scores and biochemical-related factors were identified in eighty school students aged 10–18 years.A good distribution of cognitive function was identified in approximately 56.25 % of the study population (n = 45) with a 117.52 ± 6.3 mean LOTCA-7 score. In addition to that, students with moderate (score 62–92, n = 35), and poor (score 31–62; n = 10) cognitive function were identified respectively in 43.75 % of the study population (n = 35)(Table 1). Adiposity parameters were also determined in all subjects (Table 1).School students with abnormally distributed cognitive function reported a significant increase (P = 0.05) in BMI, waist, hips, and waist- to- hip ratio (WHR) compared to normal control students.

In addition, students with moderate and poor cognitive capacity showed a little insignificant increase in the levels of both fasting sugar and glycated hemoglobin (HbA1c (%) compared to healthy controls (Table 1).

The determination of LOTCA-7 scores was reported in all participants (Table 1). Compared to control students, a significant reduction (P = 0.01) in all LOTCA 7-subset variables was observed among students with moderate to poor cognitive capacity, particularly vasomotor organization, thinking operations, attention, and concentration (Table 1).

The expression of anti-GADAs as a marker of cognitive function was determined in all school students with moderate stuttering (Fig. 2). The expression of anti-GADAs increased significantly in students with both moderate (P = 0.01) and poor cognitive (P = 0.001) scores compared to that identified in students with good cognition capacity (Fig. 2). Moreover, the expressed levels of anti-GADA antibodies correlated significantly with the reduction in the scores of cognition as measured by LOTCA 7-subset variables (Table 2). The expression of anti-GADAs antibodies in the serum of students with moderate to poor cognitive capacity, significantly (P = 0.001) correlated with the reduction in the scores of all LOTCA 7-subset variables, particularly vasomotor organization, thinking

Table 1
Participants characteristics based on cognitive capacityscores of the school students with mild stuttering (N = 80).

Parameters	Cognitive capacity(LOTCA scores) [c]			ANOVA
	Good (93–123)	Moderate (62–92)	Poor (31–62)	
N	45 (56.25)	25(31.25)	10 (12.5)	*P*
Male/Female	30/15	17/8	7/3	0.13
Age (years)	12.22 ± 1.16	12.3 ± 1.41	12.23 ± 0.51	0.316
BMI (kg/m2)	20.6 ± 1.43	22.2 ± 1.93 [a]	23.27 ± 2.32 [a,b]	0.001
Waist (cm)	67.76 ± 5.83	69.7 ± 4.35[a]	79.2 ± 6.7[a,b]	0.001
Hips (cm)	70.41 ± 5.23	72.616 ± 7.1[a]	82.12 ± 7.15[a,b]	0.001
WHR	0.69 ± 0.13	0.74 ± 0.15[a]	0.81 ± 0.12[a,b]	0.001
HbA1c (%)	3.5 ± 0.56	3.4 ± 0.56	4.02 ± 0.45	0.011
Fasting sugar	69.6 ± 2.56	75.4 ± 2.56	85.6 ± 3.45	0.021
Cognitive capacity parameters				
Orientation (O)	15.53 ± 1.98	12 ± 0.85 [a]	8 ± 0.79 [a,b]	0.001
Visual perception (VP)	22.7 ± 2.33	14 ± 1.04 [a]	9.4 ± 1.53[a,b]	0.001
Spatial perception (SP)	13.6 ± 2.54	6.9 ± 1.4 [a]	8.7 ± 1.7[a,b]	0.001
Motor praxis (MP)	10.6 ± 1.83	7.3 ± 1.15 [a]	5.8 ± 1.1[a,b]	0.001
Vasomotor organization (VO)	25.2 ± 2.1	20 ± 3.0 [a]	13 ± 0.80[a,b]	0.001
Thinking operations (TO)	24.6 ± 2.54	15.6 ± 1.14 [a]	12.4 ± 1.33[a,b]	0.001
Attention and concentration (AC)	5.3 ± 1.1	4.0 ± 1.7 [a]	2.55 ± 0.53[a,b]	0.001
Total LOTCA score	117.52 ± 6.3	79.8 ± 3.9 [a]	59.85 ± 5.34[a,b]	0.001

Values are expressed as a mean ± standard deviation. BMI, body mass index; WHR, waist-tohip ratio; BP, blood pressure; HbA1c, glycated hemoglobin; LOTCA, Loewenstein Occupational Terapy Cognitive Assessment. [a] Significant difference between high or moderate and poor activity levels. [b] Signifcantdiference between high and moderate activity levels. [c] Adjustment for multiple comparisons (Bonferroni). Significance at p < 0.05.

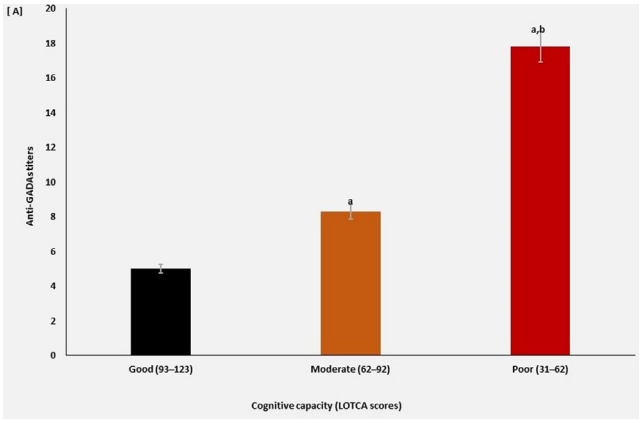

Fig. 2. Associations between the expression of serum anti-GAD antibody titers and cognitive capacity in school students with mild stuttering (n = 80). Values are expressed as mean ± SD (adjusted scores). GAD; Glutamic acid decarboxylase, LOTCA, Loewenstein Occupational Terapy Cognitive Assessment. [a] Signifcantdiference between Good or Moderate and Poor cognitive capacity (P = 0.01). [b] Signifcantdiference between Good and Moderate cognitive capacity (P = 0.001). Analysis of the data reported by covariance (ANCOVA) and adjusted for multiple comparisons by using Bonferroni.

operations, attention, and concentration compared to control students (Table 2).

In students with poor cognitive capacity, the effect of cellular inflammation and oxidative stress parameters on cognition ability was reported in this study (Fig. 3&4). A significant increase in the serum levels of both inflammatory cytokines (TNF-α, IL-6, CRP) and oxidative stress markers (TAC, NO) was reported in students of poor (P = 0.001) and moderate (P = 0.01) cognitive capacity compared to students with good cognition capacity (Fig. 3 & Fig. 4). There was a significant increase in the level of TNF-α, IL-6, and CRP in students with Poor (P = 0.001) and moderate (P = 0.01) cognitive capacity compared to those with good cognition (Fig. 3). Also, the expression of oxidative stress markers was significantly reported (Fig. 4). The levels of nitric oxide (NO) and total antioxidant capacity (TAC) significantly reduced (P = 0.001) in students with poor and moderate scores of cognitive capacity compared to those with good scores as well (Fig. 4).

The overexerted anti-GADA antibodies in students with moderate stuttering showed to be associated with increased of both cytokine and oxidative stress parameters and reflect in such way the cognition abilities of these students (Table 3).In students with moderate and poor cognitive capacity, the expression of anti-GADA antibodies correlated significantly with the increase in the levels of inflammatory markers; CRP, TNF-α, IL-6, and the decrease in the levels of both TAC and NO in students with moderate and poor cognitive capacity as shown in Table 3.

Table 2
Correlation coefficients analysis between major domains of cognitive performance (7-subsets of LOTCA scores) and the expression of serum anti-GAD antibody titers in the population of school students with mild stuttering (n = 80).

Parameters	Cognitive capacity(LOTCA scores) [c]			ANOVA *P*
	Good (n = 45) GAD Ab	Moderate (n = 25) GAD Ab [(a)]	Poor (n = 10) GAD Ab [(a,b)]	
Orientation (O)	0.16	0.41	0.25	0.001
Visual perception (VP)	0.45	0.61	0.74	0.001
Spatial perception (SP)	0.36	0.75	0.61	0.001
Motor praxis (MP)	0.24	0.92	0.28	0.001
Vasomotor organization (VO)	0.56	0.31	0.54	0.001
Thinking operations (TO)	0.27	0.45	0.38	0.001
Attention and concentration (AC)	0.36	0.38	0.47	0.001
Total LOTCA score	0.51	0.33	0.65	0.001

Data presented as coefficient (R). LOTCA, Loewenstein Occupational Terapy Cognitive Assessment. [a] Significant difference between high or moderate and poor activity levels. [b] Signifcantdiference between high and moderate activity levels. [c] Adjustment for multiple comparisons (Bonferroni). Significance at p < 0.05.

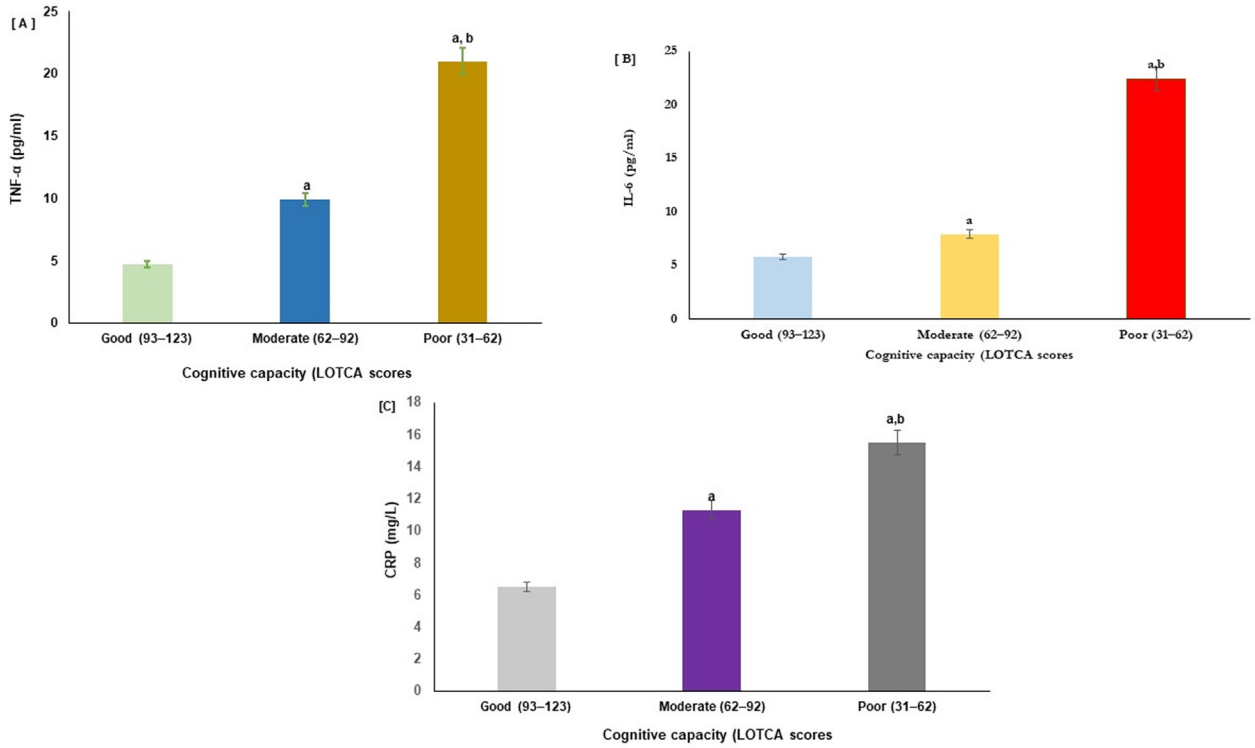

Fig. 3. Associations between serum cytokines expression (TNF-α, IL-6, CRP) and cognitive capacity in school students with mild stuttering (n = 80). Values are expressed as mean ± SD (adjusted scores). TNF-α: tumor necrosis Alpha; IL-6: interlucin-6; CRP: c-reactive protein, LOTCA, Loewenstein Occupational Terapy Cognitive Assessment. [a] Signifcantdiference between Good or Moderate and Poor cognitive capacity (P = 0.01. [b] Signifcantdiference between Good and Moderate cognitive capacity (P = 0.001. Analysis of the data reported by covariance (ANCOVA) and adjusted for multiple comparisons by using Bonferroni.

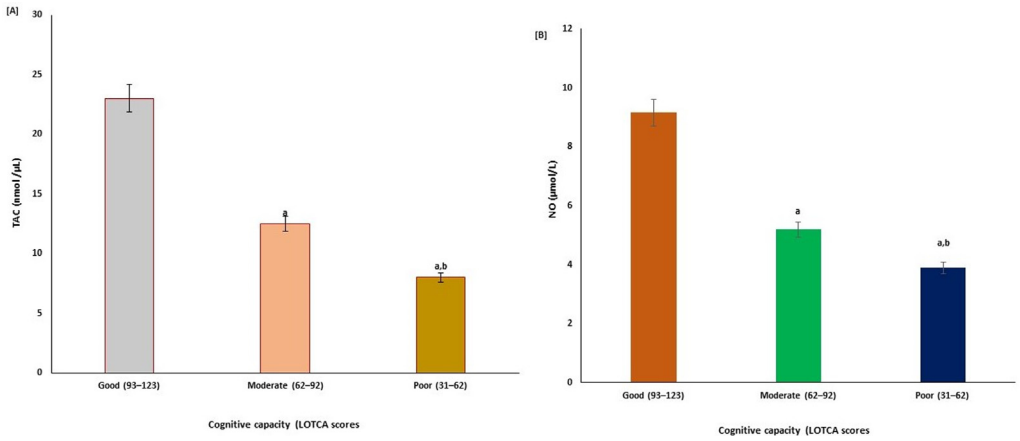

Fig. 4. Associations between serum oxidative stress parameters (TAC, NO) and cognitive capacity in school students with mild stuttering (n = 80). Values are expressed as mean ± SD (adjusted scores). TAC: total antioxidant capacity, NO: nitric oxide, LOTCA, Loewenstein Occupational Terapy Cognitive Assessment. [a] Significant difference between Good or Moderate and Poor cognitive capacity (P = 0.01. [b] Significant difference between Good and Moderate cognitive capacity (P = 0.001). Analysis of the data reported by covariance (ANCOVA) and adjusted for multiple comparisons by using Bonferroni.

4. Discussion

Previous studies reported that "some degree of decline in cognitive capacity was significantly associated with the progress of life among all ages of humans" (Moshage et al., 1995; Alghadir et al., 2022; Pieperhoff et al., 2008; Fotenos et al., 2005). "In humansin one's mid-twenties, the distortion in the biological framework that controls both thinking and reason was significantly reported" (Moshage et al., 1995; Alghadir et al., 2022; Pieperhoff et al., 2008; Fotenos et al., 2005).

In this study, cognitive capacity was measured by theLOTCA test in a total of 80 school students aged 10–18 years with moderate stuttering. Good cognitive capacity was reported in about 56.25 % of the study population (n = 45) with a 117.52 ± 6.3 mean LOTCA-7 score. However, abnormal cognitive function was identified in 43.75 % of the study population (n = 35); they were categorized into moderate (score 62–92, n = 35), and poor (score 31–62; n = 10).

A combination of various skills includingattention, learning, memory, speech and language, fine motor skills, visuospatial, and

Table 3
Correlation coefficients analysis between cytokine biomarkers (TNF-α, IL-6, CRP), oxidative stress markers (TAC, NO), and the expression of GAD antibodies titers in the population of school students with mild stuttering (n = 80).

Parameters	Cognitive capacity(LOTCA scores) [c]			ANOVA *P*
	Good (n = 45) GAD Ab	Moderate (n = 25) GAD Ab [(a)]	Poor (n = 10) GAD Ab [(a,b)]	
TNF-α (pg/ml)	0.32	0.45	0.42	0.001
Il-6 (pg/ml)	0.57	0.36	0.76	0.002
CRP (mg/L)	0.48	0.47	0.51	0.001
TAC (nmol /μL)	0.31	0.28	0.38	0.001
NO (μmol/L)	0.27	0.19	0.34	0.001

Data presented as coefficient (R). TNF-α: tumor necrosis Alpha; IL-6: interlucin-6; CRP: c-reactive protein, TAC: total antioxidant capacity, NO: nitric oxide, LOTCA, Loewenstein Occupational Terapy Cognitive Assessment. [a] Significant difference between high or moderate and poor activity levels. [b] Signifcantdiference between high and moderate activity levels. [c] Adjustment for multiple comparisons (Bonferroni). Significance at $p < 0.05$.

executive functions should be present efficiently in any individual who has good cognition. Humans with good cognitive capacity should have the ability to recognize and interpretation of the environment in the form of attention, language, memory, visuospatial, and deciding (Kruggel, 2005; Sowell et al., 2003; D'Hondt et al., 2011; Gontsana, 1998).

Thus in our study, variability in cognitive capacity was identified by the determination of LOTCA-7 scores in school students who stutters. The data showed that students with moderate to poor cognitive capacity have a significant (P = 0.001) reduction in all LOTCA 7-subset variables compared to that of cognitive good capacity. In students with moderate to poor cognitive capacity, a significant reduction (P = 0.01) in all LOTCA 7-subset variables such as thinking and concentration was observed compared to control students.

In addition, BMI, Waist, hips, WHR as parameters of obesity, and glycated hemoglobin (HbA1c (%) were reported in all participants. The data showed that students with moderate to poor cognitive capacity have a significant increase in BMI, Waist, hips, WHR, and insignificant changein glycated hemoglobin (HbA1c (%). Previous studies significantly confirmed that adolescentswith obesity were unable to do physical activities which was significantly associated with a decrease in the performance of motor functions and physical exertion tests (xxxx; Wang, 2007). Also, it was stated that cognitive capacity is significantlylinked with the change in obesity among all ages including adolescents (Wearing et al., 2006). In that research work, "students with obesity showed significant impairments in their cognitive functions, especially attention, retention, intelligence, and cognitive flexibility compared to physically active students" (Wearing et al., 2006). Their findings emphasized the need for the involvement of all school adolescent students in physical activities to minimize obesity-associated complications, including abnormity in cognitive functions (Wearing et al., 2006). In the contrary, "cognitive functionshowed no relation with to high body mass index in both healthy children and adolescents" (D'Hondt et al., 2011).

In addition, the change in sugar parameters in our students and others might be due to the influence of obesity present among students with abnormal cognitive capacity. Several studies "significantly confirmed the link of obesity withmultiple comorbidities such as diabetes, insulin resistance, and subsequent an increased risk of developing type 2 diabetes" (Meo et al., 2019; Alam et al., 2020; Wang et al., 2015).

Biological like hormonal and neurobiological changes were significantly described in varioussubjects of cognitive disabilities (Pieperhoff et al., 2008; Fotenos et al., 2005; Guh et al., 2009; Li et al., 2009; Hsu et al., 2008; Salat et al., 2004; Del Tredici and Braak, 2008; Sheline et al., 2002; Erixon-Lindroth et al., 2005; Volkow et al., 2000). In addition, the disorder in several brain metabolites has reported in young and older subjects with cognitive decline (Hsu et al., 2008). In most previousresearch, "changes in lifestyle such as adiposity, and diets were significantly linked withcognitive decline. In addition to that, alteration in biological, physiological, and biochemical systems related to brain function was reported in those with poor cognitive capacity" (Del Arco et al., 2011; Kadota et al., 2001; Debette et al., 2010; Scarmeas et al., 2009; Stine-Morrow et al., 2008; Atti et al., 2010; Whitson et al., 2010).

Previously, dysfunction of the GABAergic system was significantly reported in patients who have cognitive problems (Tinsley et al., 1997; Saidha et al., 2010; Black et al., 1998).

In this study, the expression of GAD antibodies as a marker of cognitive capacity was identified in school students with moderate stuttering.The results showed that highconcentrations of GAD antibodies were significantly determined in the sera of students with moderate to a poor cognitive capacity than those of good LOTCA 7-subset variables. In students with moderate to poor cognitive capacity, the expression of anti-GADAs correlated significantly (P = 0.001) with the reduction in the scores of LOTCA 7-subset variables. The production of GAD antibodies showed to be correlated with the changes in the cognitive parameters related to the centers of attention, concentration, thinking, organization, and orientation in the brain. Our results are in line with those who previously reported a high concentrations of GAD antibodies in association withpoor cognitive capacity (Song et al., 2009; Hofer et al., 2012; Hailpern et al., 2007; Yoon et al., 2016; Takagi et al., 2011; Alghadir et al., 2015; Chiu et al., 2008). The higher concentration of "expressed GAD might be associated with a greater impaired secretion of insulin and decreased glucose metabolism in the brain and consequently might be associated with cognitive decline by impairing the balance of inhibitory/excitatory neurotransmitters and interrupting the insulin signaling network" (Song et al., 2009; Hofer et al., 2012; Hailpern et al., 2007; Yoon et al., 2016; Takagi et al., 2011; Alghadir et al., 2015; Chiu et al., 2008).

In addition, our study evaluated the role of cytokines and biological oxidative stress on the progression of cognitive impairment among school adolescents with mild stuttering. The results of our study showed that students with moderate and poor cognitive capacity suffer from a state of higher expression of IL-6, TNF-α, and CRP, with a reduction in the expression levels of nitric oxide (NO), and TAC activity compared to those of normal or good cognitive capacity scores.

In similar to our results, "the progression of cognitive disorders including Alzheimer's disease and mild cognitive impairment showed to be linked with the excess production of cellular free radicals and in turn, increased the overall cellular oxidative stress" (Hedman et al., 2004; Mendivil et al., 2017). In addition, "worse cognitive capability showed to be associated with the decrease in the levels of blood total antioxidant capacity (TAC), elevated total homocysteine, elevated cytokines, and low physical activity levels" (Mendivil et al., 2017; Mangialasche et al., 2009; Valko et al., 2007; Ricciarelli et al., 2007; Kamel et al., 2006). Also, our results were in

accordance with those who reported,"an association with lower expression of endothelial NO in cases with cognitive decline" (Fisher and Morley, 2002; Palomar-Bonet et al., 2020; Alghadir et al., 2022; Thong et al., 2020).

Moreover, "in many brain diseases, such as cognitive decline, dementia, and Alzheimer's disease" (Manukhina et al., 2008), "the reduction in the synthesis and action of NO might be the most vital triggering factor in the diagnosis and therapy trials" (Manukhina et al., 2008). Similarly, "poor neuronal functioning with aging and other cognitive and neuropsychiatric disorders showed to be linked with overproduction of higher concentrations of cellular pro-inflammatory cytokines like IL-6 and tumor necrosis factor (TNF-α), as well as CRP" (Toda and Okamura, 2016; Talarowska et al., 2012; Dantzer et al., 2008; Castanon et al., 2015). The existence of higher concentrations of cytokines is reported to induce cognitive impairments with or without other neuropsychiatric diseases via activating pathophysiological processes (Bauer, 2005). It was confirmed previously "thatthe risk of cognitive decline, especially in the attention/executive function domain scores was significantly increased in subjects with higher concentrations of IL-6, CRP, and TNF-α respectively" (Marin et al., 2011; Tobinick, 2007; Yaffe et al., 2003).

The results of higher concentrations of cytokines and lower of antioxidant capacity and nitric oxide were significantly associated with the determined high concentrations of GAD antibodiesin all students with abnormal cognitive capacity.The expressed higher titers of anti-GADA antibodies significantly correlated with the release of serum TNF-α, IL-6, and CRP levels and a decrease in the levels of both TAC and NO among students with moderate and poor cognitive capacity. The correlation among expressed cytokines, reduced TAC, and nitric oxide like previously reported studies (Li et al., 2016; Baierle et al., 2015; Fisher and Morley, 2002; Iosif et al., 2006), confirmed the potential role of the GABAergic system in the pathogenesis and severity of thecognitive decline.

Although, this is the first of its kind study that evaluated the proposed role of the biological disorder of the GABAergic system, cytokines, and oxidative stress profiles in the pathogenesis and severity of the cognitive decline among students with mild stuttering, the influence of gender differences and their proposed effects on the cognitive decline is not fully discussed in this study. Thus, additional studies with larger cohorts are required for a more thorough evaluation of the biological effects of gender differences on cognitive capacity associated with stuttering among adolescents.

5. Conclusion

This study conclude that cognitive impairments were associated with stuttering in school students with moderate stuttering. Moreover, the abnormality of cognitive capacity in students with mild stuttering showed to be linked with higher expression of GAD antibodies, cytokines, and lower expression of endothelial nitric oxide with a reduction in the TAC activity compared to those of good or normal cognitive capacity.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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