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Using 6-CIT, P300 encephalography, and pro-inflammation assessments for screening age-related cognitive decline and exploring associated risk factors in Egyptian elderly

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Abstract

Background The elderly population is suffering from many mental health problems that are aggravated as a matter of age and cognitive decline is a serious one of them. The aim of the present work is to screen the cognitive performance among a sample of Egyptian elderly volunteers and to investigate the associated risk factors.

Results A sample of 88 elderly volunteers from both genders was enrolled in the study according to the specified eligibility criteria after signing the approval consent. Medical history and socio-demographic data were collected from all participants in addition to basic clinical examination. Cognitive performance was assessed using the 6-Item Cognitive Impairment Test (6-CIT) while the endogenous event-related potentials (ERP) was measured using P300. The inflammatory biomarkers; TNF-α and COX-2 levels were assessed in serum using ELISA technique in addition to gene expression of TNF-α, PPAR-γ and CD-36 exploration using qRT-PCR. About half (51%) of the sample under investigation showed cognitive problems with scores on the 6-CIT exceeding the normal level. TNF-α serum levels showed positive correlation with P300 latency and correlated negatively with P300 reaction time. Furthermore, serum COX-2 levels correlated positively with P300 reaction time and negatively with P300 amplitude.

Conclusion The study population is showing early signs of cognitive decline that invites attention to the importance of spreading preventive measures against further deterioration. Inflammatory biomarkers under investigation and 6-CIT are suggested to be used in prediction of early stages of cognitive decline among the elderly population.

Keywords Aging, 6-CIT, P300, Cognitive decline, Proinflammation markers, Gene expression, PPAR-γ, CD-36, TNF-α

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Background

In the few last decades, the average life span for both genders has increased to exceed the age of 65 years. In the same context, Sweed [1] is reporting the same issue for the Egyptian population which is gradually increasing in the absolute and relative numbers of the aged. The percent of older people (60 years and above) is projected to reach 20.8% in 2050 with the oldest old group (70 years and above) constituting 2.5% of the Egyptian population and 31.73% of the Egyptian elderly. Many changes are commonly witnessed in cognitive abilities as a part of normal aging that makes the elderly threatened at maintaining their functional independence with a high risk of age-associated neurodegenerative diseases [2].

Many factors attribute to cognitive problems that aggravate the negative influence of age. Some of the factors are modifiable risk factors like chronic diseases—specifically diabetes, and coronovascular problems—lifestyle behaviors (smoking, physical activity, social inclusion…), and education. On the other hand, there are non-modifiable risk factors like age, gender, race, ethnicity, and genetics [3].

Systemic inflammation, with an elevation in serum level of pro-inflammatory cytokines, is another factor highly associated with cognitive performance [4]. The pro-inflammatory biomarkers could represent another vital risk factor that contributes to the pathogenesis of cognitive decline in elderly [5]. Lately, it became evident that the brain is not immune-privileged and it can be affected by systemic inflammation including the release of pro-inflammatory cytokines such as TNF-α and COX-2 with other pro-inflammatory molecules from glial cells [6]. COX-2 is an isoform of cyclooxygenases enzyme that converts arachidonate to prostaglandins (PGEs) with crucial role in the formation of inflammatory cytokines. COX-2 is found in all areas of brain with reported role in synaptic plasticity and normal neuronal growth, however, up regulation of COX-2 has been observed in affected areas of Alzheimer's disease (AD) brain [7]. In addition, the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ) that coordinates lipid, glucose and energy metabolism is expressed at reduced levels in central nervous system (CNS) under physiological condition, but mRNA of PPAR-γ was to be highly expressed under pathological conditions such as cerebral ischemia and AD but recently was found to modulate inflammation under pathophysiological conditions as cognitive decline associated with AD [8]. Moreover, in the early phases of AD, the expression of CD-36 induced for phagocytosis purpose of β-amyloid peptides deposits (Aβ) in the brain is also linked with cholesterol and fatty acids metabolism [9, 10] and upregulation of CD-36 expression and Aβ binding were also reported to be associated with oxysterols, a byproducts of cholesterol metabolism [11]. On the other hands, Aβ deposition in the microglial cells induces pro-inflammatory cytokines production such as TNF-α, and in turn as the disease progression attained, the microglial cells maintain pro-inflammatory cytokines production and down-regulate CD-36 expression [12, 13]. Therefore, it was important to screen the expression levels of mRNA of PPAR-γ, CD-36 and TNF-α in the selected cohort of elderly volunteers and correlate their expression levels with the cognitive and/or memory status of the selected study group.

According to the most recent studies, it became greatly vital to detect and categorize subjects diagnosed with mild cognitive impairment (MCI)—in particular—since it is proved to be a symptomatic state that occurs predementia and can easily be converted to dementia if not managed properly [14]. Endogenous event-related potentials (ERP) are magnetic waves detected via encephalography where the P300 and N200 represent sensitive indicators for monitoring cognition and deterioration in terms of their latency and amplitude. As evident, the reduction in cognitive processing speed measured by ERP is associated with latency increases and amplitude decreases [15]. The Six-Item Cognitive Impairment Test (6-CIT) is another tool for screening cognitive performance. It is a neuropsychological test that gains wide popularity among specialists and consists of six simple questions investigating attention and working memory, space and time orientation, and delayed recall [16].

Consequently, the main aim of the present work is to screen cognitive abilities among a random sample of elderly volunteers in Egypt and to investigate the associated risk factors. This could represent a supportive guide for healthcare providers and decision-makers for settlement of preventive measures that may delay or prevent the onset of possible brain diseases and/or cognitive debilitations.

Methods

Participants

Elderly volunteers from both genders above ages of 45 years for women and 55 years for men were enrolled in the study and recruited at the outpatient clinic in the Centre of Excellency, National Research Centre (NRC), Egypt. Subjects were visiting the clinic in response to an open call for general checkup and early detection of cognitive decline manifestations that extended for 3 months (starting at October 2019). Such an initiative was introduced through an internally funded project by the NRC.

Each volunteer received detailed explanation about the aim of the project and the benefit versus risks. Once the volunteer accepts joining the study, he\she was asked to sign an informed written consent. The whole

methodology of the study has been performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and was approved by the ethical committee of the NRC (No. 19-225, 2019). Basic information including socio-demographic data and medical history were filled using a customized questionnaire by the clinical team, followed by a general and neurological clinical examination by the physicians. Volunteers who declared any experience of neurological disease such as epilepsy, transient ischemic attacks, neurodegenerative disease or severe neurological impairment due previous condition were excluded from the study.

Assessment of cognitive abilities

The 6-Item Cognitive Impairment Test (6-CIT) Kingshill Version 2000® [17] was used as dementia screening tool for all volunteers ($n=88$). The 6-CIT questionnaire comprised six questions where one of them targets memory and the test taker is asked to remember a 5-item address (Q3). Two are for calculation and include reciting months of the year backwards and telling numbers from 20 to 1 backwards (Q 6, 5). Three questions are testing orientation by asking about the year, month, and time of day at the time of examination (Q1, 2 and 4). The 6-CIT is completed in approximately 2 min and has shown a big deal of acceptability among experts and professionals. A score <7 indicates normal state, from 8–9 indicates mild cognitive impairment while scores >9 denote significant cognitive decline [18].

Endogenous event-related potentials (ERP) was measured using encephalography through the classic P300 for 20 participants. The ERP approach—as an instrument of neurophysiological examination—is currently used for assessing complicated brain processes by recording potentials reflecting the intrinsic brain activity with changes in potentials. The P300 cognitive evoked potential with recording selective attention to a stimulus is used to study mechanisms of mental disturbances, in reticulothalamic systems, limbic and neocortical mechanisms and short-term memory. Both P300 latency (related to information processing time), amplitude (related to attention level), and Reaction time to stimuli (RT) (related to the ability to detect, process, then respond to a stimulus) were detected. Participants were instructed to discriminate the rare stimulus while irrelevant stimuli are also being presented. Tasks were auditory with stimuli at different sound frequencies [19]. Test was carried up at the Clinical Neurophysiology Unit, kasr El Ainy Hospital, Egypt.

Biochemical assessments

Fasting blood samples (4–5 ml) were collected from all participants. The blood samples were withdrawn by venipuncture. The serum was separated and stored at −20 °C until biochemical analysis. TNF-α and COX-2 levels in serum were analyzed using commercial ELISA kits (Elabscience, USA). The assays were performed according to the manufacturer's protocols.

Gene expression analysis

RNA extraction

One ml of whole blood was withdrawn from each subject in a sterile vacutainer 5-ml tube containing EDTA as anticoagulant agent. Blood samples were stored on ice and processed within 30 min after the collection. Total RNA was extracted from whole blood [20] of each sample using TRIzol-based method (Ambion, Life Technologies, CA), according to manufacturer's instructions. RNA concentration and purity were monitored for each sample using nanodrop Spectrophotometer device (2000 C; Thermo Scientific, USA). RNA samples were immediately stored at −80 °C until further processing to avoid RNA degradation.

Quantitative reverse transcription-polymerase chain reaction

The reverse transcription reaction was performed from 2 µg of total RNA using Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Lithuania), according to manufacturer's instructions using SimpliAmp Thermal Cycler (applied biosystems, Thermo Fisher Scientific, Singapore). Gene expression of CD-36, PPAR-γ, and TNF-α were carried out using the Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific, Lithuania), according to the standard procedures. Briefly, in a 25-µl reaction volume, 5 µl complementary DNA was added to 12.5 µl SYBR Green mixture, 5.5 µl RNase free water, and 2 µl of each primer (5 pmol/µl). The primer sequences used in the present study are listed in Table 1 [21–23]. The thermal cycler protocol consisted of 40 cycles of denaturation for 15 s at 95 °C, annealing for 60 s at 60 °C, and extension for 60 s at 72 °C using the

Table 1 The primer sequences used for real-time polymerase chain reaction

Gene	Primer sequence
PPARγ	F5′-CGACCAAGTAACTCTCCTCA-3′ R-5′-GTTCGTGACAATCTGTCTG-3′
TNF-α	F-5′-CTGAACTTCGGGGTGATCG-3′ R-, 5′-GCTTGGTGGTTTGCTACGAC-3′
CD-36	F 5′-AAGTCACTGCGACATGATTAATGG-3′ R 5′-GAACTGCAATACCTGGCTTTTCTC-3′
B-actin	F-5′-AACTGGAACGGTGAAGGTGAC-3′ R-5′-TGTGGACTTGGGAGAGGACTG-3′

PPAR-γ: peroxisome proliferator-activated receptor-γ; TNF-α: tumor necrosis factor-α

quantitative real-time PCR (qRT-PCR) (DNA Technology, Moscow, Russia). β-Actin was used for normalization of mRNA levels.

Statistical analysis

Data were computed using SPSS 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBMCorp), and included descriptive (mean (SD) and frequencies) and Pearson test for correlation. Values with p value less than 0.05 were considered significant.

Results

Socio-demography and medical history

In the three months' time of the study, 88 volunteers took the 6-CIT test and completed the related investigations. Some of the volunteers' data were missing but did not affect the results. The mean age of the sample was 57 years ranging from 48 to 77 years. The socio-demography and medical history are shown in Table 2.

Table 2 Descriptive data of study sample represented in frequency percentages

Variables (*n*)		Frequency (%)
Age (77)	≤ 55	35 (46)
	56–65	33 (43)
	> 65	9 (11)
Gender (80)	Male	23 (29)
	Female	57 (71)
Occupation (77)	Employee	45 (58)
	Worker	9 (12)
	Researcher	4 (5)
	Retired	2 (3)
	House-wife	17 (22)
Marital Status (77)	Married	65 (84)
	Divorced	7 (9)
	Widow	5 (7)
Smoking (80)	No	72 (90)
	Yes	8 (10)
Obesity (72)	No	5 (7)
	Yes	67 (93)
Chronic diseases (CD) (79)	No	26 (33)
	Yes	53 (67)
Type of CD (53)	Hypertension	16 (30)
	Diabetes mellitus (DM)	12 (23)
	Hypertension and DM	13 (24)
	Others	12 (23)
Family history of Alzheimer (79)	No	72 (91)
	Yes	7 (9)
Family history of dementia (79)	No	74 (94)
	Yes	5 (6)
Cognitive decline (81)	Normal (≤ 7)	40 (49)
	Mild (8–9)	14 (17)
	Significant (> 9)	27 (34)

CD: chronic diseases

Assessment of cognitive abilities

Table 3 shows the results of 6-CIT total score and those for all the questions. The mean value for the total score (7.3) is slightly above the normal upper limit (7) with an indication that the sample could be at border line of showing signs of cognitive decline. Most of the study sample found some difficulty in question 6 (saying months in reverse), question 5 (counting numbers back), and in question 3 (remembering the memory phrase).

Table 4 shows the ERP P300 test results with an illustration of the number of participants which were subjected to the test and their total score on the 6-CIT (8.9 with minimum and maximum scores for each item in the studied sample ($n=20$). In Table 5, it could be noticed that counting back from 20 to 1 was the best question that was able to discriminate between the three levels of cognitive state since it showed significant difference between each two groups indicating good sensitivity. Participants suffering from significant cognitive decline showed significant higher scores on repeating the memory phrase, stating the time, counting numbers backwards and saying months in reverse compared to the normal group. While they showed significant higher scores in stating the time and counting numbers backwards compared to the mild state group [24].

Biochemical and gene expression measurements

Tables 3 and 4 show the results of the serum biochemical markers; TNF-α,COX-2 as mean values and gene expression levels of TNF-α, PPAR-γ and CD-36 expressed as median with minimum and maximum fold gene increase values (Table 6).

The scores on the 6-CIT and P300 ERP test have been tested for correlation with the investigated modifiable (chronic diseases, BMI, TNF-α, COX-2 protein levels) and non-modifiable (age) variables and results are represented as shown in Tables 7 and 8, respectively. Highly significant correlations at $p<0.01$ were also witnessed between TNF and PPAR-γ, TNF and CD-36, and between PPAR-γ and CD-36 with R values of 0.703, 0.29 and 0.48, respectively. A positive correlation is also shown between the amplitude and the presence of chronic diseases.

Discussion

Aging in the Egyptian population is a phenomenon with many socio-economic impacts and health consequences and is rendered as a public health challenge

Table 3 Descriptive data of the biochemical markers and the 6-CIT total score and its individual items represented as mean and standard deviation

	Parameter	*n*	Minimum	Maximum	Mean ± SD
Biochemical markers	TNF-α (pg/ml)	81	7.3	17.6	7.6 ± 1.1
	COX-2 (pg/ml)	86	8.3	25.6	10.6 ± 1.9
6-CIT	Total score	81	0	14	7.3 ± 3.4
	Q1. What year is it?	81	0	4	0.05 ± 0.4
	Q2. What month is it?	81	0	3	0.07 ± 0.5
	Q3. Repeating a memory phrase	81	0	10	1.1 ± 2.5
	Q4. What time is it?	81	0	4	0.3 ± 1
	Q5. Count back from 20 to 1	81	0	4	1.5 ± 1.7
	Q6. Say months in reverse	81	0	10	4.3 ± 2.3

TNF-α: tumor necrosis factor-α; COX-2: cyclooxygenase-2; 6-CIT: 6-Item Cognitive Impairment Test

Table 4 Descriptive data of the biochemical markers and the EEG P300 test outputs represented as mean and standard deviation for the 20 cases that performed the test

	Parameter	*n*	Minimum	Maximum	Mean ± SD
Markers	TNF-α(pg/ml)	16	7.3	10.5	7.7 ± 0.8
	COX-2(pg/ml)	16	8.3	25.6	11.8 ± 3.6
6-CIT	6-CIT-total score	15	4	14	**8.9 ± 3**
ERP P300	P300 latency	20	178	417	309.0 ± 58.1
	Amplitude	19	4	27	13.9 ± 6.6
	Response to stimulus time	19	213	762	431.9 ± 121.7

Significant or highly significant value is in bold

6-CIT: 6-Item Cognitive Impairment Test; ERP: endogenous event-related potentials

Table 5 Comparing means between participants with normal, mild and significant cognitive decline regarding the individual items of the 6-CIT

6-CIT items	Normal	Mild	Significant
Q1. What year is it?	0.0 ± 0.0	0.3 ± 1.0	0.0 ± 0.0
Q2. What month is it?	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.8
Q3. Repeating a memory phrase	0.5 ± 1.2[a]	1.6 ± 3.3	2.2 ± 3.5[a]
Q4. What time is it?	0.0 ± 0.0[a]	0.0 ± 0.0[b]	0.9 ± 1.5[a b]
Q5.Count back from 20 till 1	0.6 ± 1.1[a]	1.3 ± 1.4[b]	2.6 ± 1.7[a b]
Q6. Saying months in reverse from December till January	3.0 ± 1.9[a]	4.8 ± 3.0	5.3 ± 2.3[a]
Total Score	4.2 ± 1.9	8.0 ± 0.0	11.2 ± 1.3

6-CIT: 6-Item Cognitive Impairment Test

[a] Significant difference between normal and significant cognitive decline groups at $p < 0.05$

[b] Significant difference between mild and significant cognitive decline groups at $p < 0.05$

that threatens the economic development. The old-age dependency ratio keeps on growing where the number of individuals at retirement is increasing with a higher rate than that of individuals at working ages. Cardiovascular and cerebrovascular diseases, as well as Alzheimer's disease are expected to increase day after day. Profound data on elderly characteristics became highly essential as a first step to explore the actual situation of the aged population in order to avail development planning in a proper manner [1].

Frailty is an evolving concept that is recently used in clinical practice and epidemiological research in relation to aging health consequences. It is not yet defined in terms of definition or diagnosis yet it is related to a broad perspective that includes psychological, cognitive, nutritional, and social factors. Cognition is a main component of frailty due to aging that could be enhanced by exposure to education and exhibiting complex life-long activities. Diet-related factors like sedentary lifestyle and lower food consumption that leads to protein-energy malnutrition are important contributors to the progression of frailty [25]. Many helpful actions and new technologies are now available that may secure a better state of living for aged people. Ambient assistant living (AAL) is one technique of the new consumer health informatics technologies (CHI) that aims to improve the comfort, quality of life, and safety of the old population by integrating technology into their lives to make them more self-determined. Serious games for healthcare are another

Table 6 Testing the correlation between fold change of TNF-α, PPAR-γ and CD-36

Gene expression	Fold change TNF-α	Fold change PPAR-γ	Fold change CD-36
n	88	88	88
Median (min, max)	1.2537 (0.014, 381.87)	0.8965 (0.002, 162.28)	0.6082 (0.002, 76.07)
R (P)	0.703 (0.0000)[a]	0.290 (0.008)[b]	0.483 (0.000)[c]

TNF-α: tumor necrosis factor-α; PPAR-γ: peroxisome proliferator-activated receptor-γ

[a] Statistically significant correlation between TNF and PPAR

[b] Statistically significant correlation between TNF andCD-36

[c] Statistically significant correlation between PPAR and CD-36

CHI that train elderly on achieving behavioral changes as rehabilitation measures [26].

The 6-CIT is a well-recognized screening tool for mild cognitive impairment (MCI) and is now recommended to be used as an online self-screening tool as declared in many research articles [18]. According to the study results, 51% of the sample are experiencing cognitive problems (mild (17%) and significant (34%) cognitive decline according to their scores on the 6-CIT. This percentage could represent an alarm that invites serious attention if compared to data witnessed among other populations. In a similar study performed on a sample in Portugal with mean age of 88.9 ± 8.8 years, more than half of the sample ($n=44$) did not show mild cognitive impairment [25]. Another longitudinal study was performed on African and White Americans residing in Chicago within a geographically defined community. Among the 1168 individuals above the age of 65 who were included in the study, 395 only were diagnosed for MCI and 159 witnessed other kinds of cognitive impairments [27].

As mentioned by Ball et al. [28], diabetes and poor cardiovascular health could be a leading cause to cognitive impairment as it is associated with higher incidence of stroke. The author also states that some studies reported better speed of processing performance and reasoning for older adults free from cardiovascular problems. In the present work, 77% of the sample is experiencing hypertension, diabetes mellitus or both yet with no direct impact on cognitive performance. On the contrary, negative association is recorded among the study sample between chronic diseases and between the 6-CIT total score as well as the P300 latency. Despite unexpected yet many other studies are showing similar findings [25]. Some other studies report gender differences in ERP values which is not the case in the present work [29, 30].

The P300 latency which is related to information processing time and amplitude which is related to attention level are known to be affected by age, where latency increases and amplitude decreases. Both parameters also show significant difference in values as compared between normal individuals, patients with mild cognitive impairments and those suffering from Alzheimer's disease [31]. From this perspective, a growing interest is focusing on exploring the ERP parameters to be further used in prediction and diagnosis of age-related cognitive impairments [32]. Pavarini et al. [15] in their review article showed values for P300 latency obtained from many studies to range between 320 and 479 ms among healthy elderly while amplitude ranged between 2.2 and 18.5 μV with no pattern of response to the stimuli. This big variation among results is due to differences in methodology and/or sample characteristics. Broadly speaking, many factors are contributing to the ERP outputs that make it a big challenge to standardize a valid index in relation to its values. Alternatively, it is suggested to compare individual values obtained in a single study to obtain more convenient interpretation of data analysis. In the present study, data showed negative correlation between P300 latency and age with non-significant p-value which is similar to findings obtained from the 6-CIT. Both results suggest the detected cognitive impairment to be a consequence of factors other than age. According to Lucci et al. [33], the reaction time to the stimuli in elderly showed a mean of 555 ms compared to 480 ms in the young. In the present work, mean reaction to stimulus time is 431.9 ± 121.7 ms that is even lower than values detected in the young population.

Neuro-inflammation is proved to be an early stage marker of pathogenesis in cognitive decline. Increased levels of inflammatory biomarkers are witnessed in both plasma and CNS in patients with Alzheimer's disease [34]. Underlying mechanisms of systemic inflammation pathogenesis include damaging and crossing the blood–brain barrier (BBB) that cause triggering of some brain-specific inflammatory response like increasing the rate of apoptosis, decreasing synaptic function, inhibiting neurogenesis resulting in neuronal death [35]. Systemic pro-inflammatory cytokines can disrupt the BBB by manifesting structural changes like tight junction dysfunction,

Table 7 Testing the correlation between 6-CIT and its domains and cognitive decline risk factors: TNF-α, COX-2, age, BMI and chronic diseases

		TNF-α	COX	Age	BMI	CD	TS	Q1	Q2	Q3	Q4	Q5	Q6
TNF-α	***r***	1											
	p												
	N	89											
COX	*r*	−0.080	1										
	p	0.457											
	N	89	89										
Age	*r*	0.044	−0.068	1									
	p	0.722	0.585										
	N	67	67	72									
BMI	*r*	−0.013	−0.098	**−0.324****	1								
	p	0.917	0.429	**0.010**									
	N	67	67	**63**	71								
CD	*r*	0.075	−0.173	0.076	0.003	1							
	p	0.528	0.140	0.540	0.983								
	N	74	74	67	65	79							
TS	*r*	−0.039	−0.033	0.032	−0.176	−0.206	1						
	p	0.739	0.775	0.794	0.152	0.078							
	N	76	76	69	68	74	81						
Q1	*r*	−0.016	−0.051	−0.111	0.027	0.081	0.024	1					
	p	0.890	0.658	0.364	0.825	0.492	0.832						
	N	77	77	69	68	74	81	82					
Q2	*r*	−0.039	0.119	0.126	−0.082	−0.063	0.175	−0.018	1				
	p	0.741	0.306	0.303	0.506	0.597	0.118	0.875					
	N	76	76	69	68	74	81	81	81				
Q3	*r*	−0.067	−0.116	−0.099	0.193	−0.126	**0.329****	−0.048	0.062	1			
	p	0.565	0.318	0.421	0.115	0.284	**0.003**	0.670	0.583				
	N	76	76	69	68	74	**81**	81	81	81			
Q4	*r*	0.167	−0.066	0.174	−0.195	−0.065	**0.412****	−0.035	0.118	−0.134	1		
	p	0.150	0.569	0.153	0.110	0.584	**0.000**	0.757	0.294	0.233			
	N	76	76	69	68	74	**81**	81	81	81	81		
Q5	*r*	−0.048	−0.007	0.227	**−0.375****	−0.144	**0.526****	0.035	0.050	**−0.262***	**0.286****	1	
	p	0.679	0.955	0.060	**0.002**	0.223	**0.000**	0.756	0.659	**0.018**	**0.010**		
	N	76	76	69	**68**	74	**81**	81	81	**81**	**81**	81	
Q6	*r*	−0.027	0.072	−0.087	−0.106	−0.036	**0.537****	−0.113	−0.091	**−0.337****	0.115	0.199	1
	p	0.815	0.538	0.478	0.390	0.763	**0.000**	0.315	0.417	**0.002**	0.307	0.074	
	N	76	76	69	68	74	**81**	81	81	**81**	81	81	81

Significant or highly significant values are in bold

Q1: What year is it?, Q2: What month is it?, Q3: Repeating a memory phrase, Q4: What time is it?, Q5: Count back from 20 till 1, Q6: Saying months in reverse

CD: chronic disease; BMI: body mass index; TS: total score

*Correlation is significant at the 0.05 level (2-tailed), ** correlation is highly significant at the 0.01 level (2-tailed)

r correlation coefficient, *P* probability, *N* number

pericyte dysfunction, and increased oxidative stress in endothelial cells. Neuro-inflammation is then augmented by the activation of hypothalamic cytokine receptors [36] with direct negative effect on the hypothalamic related functions that influence feeding, stress regulation, metabolism, and cardiovascular function [37].

According to King et al. [37], inflammation could be part of the onset of cognitive decline in patients with MCI related to AD and anti-inflammatory medications could help at the earliest stages of neurodegenerative diseases. Cacabelos et al. [38] found serum TNF-α levels to be significantly lower in AD with the value of 2.5 ± 1.25 pg/

Table 8 Testing the correlation between P300 variables and cognitive decline risk factors: TNF-α, COX-2, age, BMI and chronic diseases

Parameters		P300 latency	Amplitude	RT	TNF-α	COX-2	Age	BMI	CD
P300 latency	*r*	1							
	p								
	n	20							
Amplitude	*r*	−0.381	1						
	p	0.108							
	n	19	19						
RT	*r*	−0.250	−0.008	1					
	P	0.303	0.974						
	n	19	18	19					
TNF	*r*	0.276	0.023	−0.068	1				
	P	0.301	0.935	0.810					
	n	16	15	15	89				
COX	*r*	−0.339	−0.483	0.208	−0.080	1			
	P	0.199	0.068	0.458	0.457				
	n	16	15	15	89	89			
Age	*r*	−0.609*	0.016	0.513	0.044	−0.068	1		
	P	0.021	0.955	0.073	0.722	0.585			
	n	14	14	13	67	67	72		
BMI	*r*	0.426	0.028	−0.431	−0.013	−0.098	**−0.324****	1	
	P	0.128	0.924	0.141	0.917	0.429	**0.010**		
	n	14	14	13	67	67	**63**	71	
CD	*r*	−0.503	**0.576***	0.309	0.075	−0.173	0.076	0.003	1
	P	0.056	**0.031**	0.282	0.528	0.140	0.540	0.983	
	n	15	**14**	14	74	74	67	65	79

Significant or highly significant values are in bold

RT: reaction to stimulus time; TNF-α: tumor necrosis factor alpha; COX-2: cyclooxygenase enzyme; BMI: body mass index; CD: chronic diseases

*Correlation is significant at the 0.05 level (2-tailed), ** correlation is highly significant at the 0.01 level (2-tailed)

ml at $p < 0.01$ than in the control group (10.66 ± 8.92 pg/ml). They also figured out a negative correlation between serum TNF-α levels and age in AD but not between serum TNF-α levels and mental performance or cerebrovascular risk in AD patients. The authors concluded the decrease in serum level of TNF-α in AD to be poorly related to cognitive dysfunction and/or neurovascular damage, and suggested it to be just reflecting an endogenous immune deregulation. In another research paper, King et al. [39] stated greater severity of cognitive impairment to be associated with lower levels of serum TNF-α and they came to a conclusion that inflammatory markers decrease with disease progression. Similarly, in the present study TNF-α showed negative correlation with the 6-CIT total score but most of the individual items yet results are insignificant. Additionally, the TNF-α serum levels showed positive correlation with P300 latency but negative correlation with P300 (retention time) in the group that performed the ERP test, yet results are insignificant. Even results are insignificant this could represent an alarm that the study sample are witnessing signs of pathogenesis and must follow preventive measures against a worse state of mental fitness. Interestingly, the mean value of 6-CIT of group performed ERP showed they are at the borderline of significant cognitive decline, while the mean 6-CIT value for the whole sample showing them to be at the early stage of mild impairments (Table 4).

In the present work, COX-2 levels in plasma were also assessed (Table 3), COX-2 levels showed negative correlation with the 6-CIT total score which is in accordance with TNF-αresults, as well as negative correlation with P300 amplitude but positive correlation with P300 RT (reaction to time stimuli).Even results are not statistically significant, it points out that COX-2 enzyme could be associated with assessment parameters measuring cognitive decline and this is in agreement with previous studies on the effect of neuronal overexpression of COX-2 on developing deficit in memory, and changes in behavior [40].

For more confirmation, other related signaling markers in the pro-inflammatory molecular pathway were

measured in blood samples of volunteers using qRT-PCR techniques. The nuclear receptor PPAR-γ, a well-defined receptor in modulating inflammation and immune system, and an upstream molecular signal regulator of the TNF-α expression through NF-$_k$β pro-inflammatory signaling pathway. The CD-36scavenger receptor expressed on microglia cell surface plays a pivotal role in inflammation by clearance and engulfing of Aβ plaques is also a direct target gene of PPAR-γ receptor [41]. All genes were assessed as fold change in mRNA gene expression. Results are represented and shown in Table 6, where TNF-α mRNA gene expression results were correlated with 6-CIT total score, P300 amplitude and RT and results (data not shown, see Additional file 1) showed similar correlation with cognitive parameters as those obtained with TNF-α serum level, thus confirming TNF-α results at both gene expression and protein levels.

Interestingly there was a highly significant positive correlation between TNF-α, PPAR-γ and CD36 genes expression pattern as shown in Table 6, supporting the role of PPAR-γ -CNS levels as it was reported to be highly expressed in conditions such as cerebral ischemia and AD [8]. Moreover, CD-36, for β-amyloid peptides (Aβ) phagocytosis, was reported to be highly expressed in the early phases of AD while it is down-regulated as progression of the disease (12;13). Our observation suggests a possible strong association between those inflammatory markers in prediction of early stages of cognitive decline during clinical studies, an observation that needs to be thoroughly studied in future researches.

Conclusion

In conclusion, cognitive impairments and neurodegenerative consequences of age represent an actual challenge for all nations in light of the rapid advancements in health services and the life span average that keeps in growing, and Egypt is not apart from the issue. More than half of the present study sample showed scores of mild or significant cognitive decline with a mean total score on the 6-CIT that exceeded the normal cutoff. TNF-αand COX-2 serum levels suggest the study population could be at an early stage of the threat of cognitive decline. This invites serious attention and further studies for better exploration of the cognitive status of the elderly population in Egypt in view of neuroinflammatory disorder pathogenesis. Life style that guarantees sustained social interaction, physical exercise, reading and mental exercising, attending cultural events, continuing education, and intact family relationships are proved to be related to better cognitive functioning with age. It is highly recommended to raise campaigns that spread awareness of the proper measures for early prediction of age-related health deteriorations including the cognitive decline and how it could be managed. Offering screening investigations through the governmental special care for early diagnosis of non-communicable diseases as part of the adopted influential health initiatives all over the governorates is highly recommended to save the elderly from the inevitable health consequences of age. According to the present work, 6-CIT and serum as well as gene expression levels estimation of pro-inflammatory biomarkers could be of great help for the early detection of cognitive decline.

Abbreviations

6-CIT	6-Item Cognitive Impairment Test
AAL	Ambient assistant living
AD	Alzheimer's disease
Aβ	β-Amyloid peptides deposits
BBB	Blood–brain barrier
BMI	Body mass index
CD	Chronic diseases
CHI	Consumer health informatics technologies
CNS	Central nervous system
COX-2	Cyclooxygenase-2
ERP	Endogenous event-related potentials
MCI	Mild cognitive impairment
PGEs	Prostaglandins
PPAR-γ	Peroxisome proliferator-activated receptor-γ
RT	Reaction time to stimuli
TNF-α	Tumor necrosis factor-α

Supplementary Information

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Additional file 1: S1. Testing the correlation between P300 variables, 6-CIT scores and cognitive decline risk factors; Fold Change TNF-α, Fold Change PPAR-γ, & Fold Change CD-36.

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Author contributions

All authors contributed to the study conception and design. N.A.M., and S.F.S. recruited eligible participants and conducted general and neurological examination. M.S.S. was responsible for cognitive examination. A.F.G. conducted biochemical analyses. H.M.Z. and A.H. were responsible for molecular study. H.R.E. performed P300. M.S.S. and O.A.H. analyzed the data and wrote the main manuscript. Authors A.F.G., N.A.M., S.F.S. and H.M.Z. contributed to writing and reviewing the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The data sets generated and analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Approval was granted by the Ethics Committee of The National Research Centre (No. 19-225, 2019); all participants provided written informed consent and the study was performed in accordance with the Declaration of Helsinki guideline.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

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