



Manuscript ID ZUMJ-2111-2419 (R2)
DOI 10.21608/zumj.2021.107835.2419

ORIGINAL ARTICLE

The relationship between high sensitive c- reactive protein, interleukin-6, tumor necrosis factors - α and cognitive function in elderly diabetics in Zagazig University Hospitals

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Submit Date 2021-11-29
Revise Date 2021-12-23
Accept Date 2021-12-31

ABSTRACT

Background: Cognitive dysfunction, including mild cognitive impairment and dementia, is considered as an important comorbidity and complication of diabetes and associated with inflammatory markers. Higher serum levels of CRP, IL-6 and TNF- α are associated with deteriorating cognitive function in elderly. The goal of this research was to detect cognitive impairment in elderly individuals with diabetes and their relation to hs-CRP, IL-6 and TNF- α in those individuals. Subjects and methods: two hundred fifty-five elderly individuals which were divided into 3 groups according to present or absence of diabetes together with level of diabetes control; Group I: 85 elderly individuals without diabetes, Group II: 85 elderly patients with controlled diabetes. Group III: 85 elderly patients with uncontrolled diabetes. Routine laboratory investigations together with lipid profile, HbA1C, FBG, 2hpp, hs- CRP, IL-6 and TNF- α were analyzed in three groups using blood serum sample then correlated to degree of cognitive impairment using mini mental state examination (MMSE). Results: the present study proved that a statistically significant negative correlation between hs-CRP and MMS score examination in total population was found $P < 0.00001$, there is statistically significant negative correlation between hs-CRP, IL-6, TNF- α and MMS score examination in total elderly subjects with diabetes $P < 0.001$. As well, there is significant positive correlation between hs-CRP and FBG $p < 0.001$. Conclusion: estimation of inflammatory markers in elderly patients with diabetes may be a reliable indicator to identify and anticipate cognitive dysfunction in such individuals.

Keywords: hs-CRP, IL-6, TNF- α , cognitive function, elderly diabetics.

INTRODUCTION

The number of older adults who are type 2 diabetics is increasing. Hypoglycemia is a frequent adverse effect of treatment with oral hypoglycemics or insulin in older adults with diabetes⁽¹⁾. Both macro and micro vascular complications occur with higher frequency in geriatric diabetics⁽²⁾. T2DM, characterized by insulin resistance or insulin dysfunction might

enhance cognitive dysfunction⁽³⁾. Diabetes is associated with decreasing in cognitive abilities and greater cognitive dysfunction among older women, Cognitive dysfunction should be considered as important part of chronic complications of diabetes⁽⁴⁾. Understanding the pathways for deterioration of structural integrity of the brain could find microvascular causes,

amyloidogenic effects, or other not-yet-known effects of diabetes mellitus ⁽⁵⁾.

The Mini Mental State Exam is a tool used to systematically assess mental status. The full score is 30 and a score of 23 and lower suggests cognitive impairment so it is practical to be repeated ⁽⁶⁾. Many studies have stated that there is an association between inflammatory markers and the risk of dementia. Higher serum levels of inflammatory markers are accompanied with decreased cognitive abilities in elderly ⁽⁷⁾. Many patients (especially elderly) with diabetes mellitus have higher risk of cognitive decline. Elevated hs-CRP, IL-6 and TNF- α are a risk factor for development of DM and have significant role in cognitive dysfunction ⁽⁷⁾. So the goal of this work is to study the correlations between cognitive dysfunction and the level of hs-CRP, IL-6 and TNF- α .

SUBJECTS AND METHODS

Comparative cross-sectional study was carried out in Internal Medicine Department, Faculty of Medicine, Zagazig University Hospitals between December 2018 to December 2019. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Selection of cases: By confirmation of diabetes by taking thorough history from patients together with accurate laboratory data (diagnosis confirmed by A1C criteria or plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-h plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT)) ⁽⁸⁾.

Group I: Elderly without diabetes group: 85 subjects 53 of them were males (62.4%) and 32 of them were females (37.6%), their ages were ranged from 66 – 88.

Group II: Patients with controlled diabetes on oral hypoglycemic drugs: 85 individuals, 51 of them were males (60%) and 34 of them were females (40%), their ages were ranged from 65 – 88 years.

Group III: Patients with uncontrolled diabetes on oral hypoglycemic drugs: 85 individuals 49 of them were males (57.6%) and

36 of them were females (42.4%), their ages were ranged from 66 – 91 years.

Inclusion criteria: Age: > 65 years and of either sex, patient with type 2 diabetes mellitus on oral hypoglycemic drugs and control group (healthy population as evidenced clinically and by investigations).

Exclusion criteria: Any patient with insulin dependent either type1 or type2 DM, hepatic, renal, pulmonary disease or hypertension or obesity must be excluded.

Ethical clearance: Written Informed consent was taken from the patients or patients' relatives to participate in the study. Approval for performing the study was obtained from Institutional Review Board (IRB) approval.

Operational design: All subjects of the study were subjected to:

- Full precise history and detailed clinical examination as well as drug prescriptions. General examination and Local examination of different systems with thorough neurological examination.
- Routine investigations were done according to protocol of clinical pathology and laboratories of Zagazig University Hospital: After fasting for 12 h at least, 10 ml of blood was taken as sample for Complete blood count (CBC): including platelet indices. ESR. Liver function tests. Kidney function tests. Bleeding profile. Fasting blood glucose level. Lipid profile, including total cholesterol, LDL-c, HDL-c, triglycerides.
- **Special investigations:** Highly sensitive C-reactive protein (hs-CRP) normal range (up to 3 mg/l), Interleukin -6 (IL-6) normal range (0 -16.4 pg/ml) and tumor necrosis factor alpha (TNF- α) normal range (0 -8 pg/ml)

Specimen type: Blood serum

Technique used: ELISA

Assessment of cognitive function using mini mental state examination (MMSE): There are three cut-off levels were applied to classify the degree of cognitive impairment: no cognitive impairment=24-30; mild cognitive

impairment=18-23; severe cognitive impairment=0-17⁽⁹⁾.

Statistical analysis

All data were analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, Illinois, USA), MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; <https://www.medcalc.org>; 2015).

Continuous variables were expressed as the mean ± SD, median and range while the categorical variables were expressed as a number (percentage). Continuous variables were checked for normality by using Shapiro-Wilk. One-Way ANOVA was used to compare normally distributed variables in three groups. Kruskal-Wallis H (KW) test was used to compare non-normally distributed variables in three groups. Percent of categorical variables were compared using the Chi-square (χ^2) test.

Pearson product-moment correlation coefficient was used to assess correlation between MMS and study parameters if data is parametric $p < 0.05$ was considered statistically significant (S).

Post-hoc Fisher's Least Significant Difference test (LSD) tests were used according to homogeneity of variances.

RESULTS

There is statistically significant difference as regard MMS between group I, group II and group III. $P < 0.001$ (Table 2)

There is statistically significant difference as regard hs-CRP between different studied groups I, II and III $P < 0.00001$ (Table 3).

There is positive correlation between hs-CRP and FBG $P < 0.001$, A1C and total cholesterol levels in total population ($n=255$) and it is statistically significant (Figure 1).

There is statistically significant correlation and it was negative between hs-CRP and MMS score examination in total population ($n=255$) $P < 0.00001$ (Figure 2).

There is negative correlation between hs-CRP IL-6, TNF- α and MMS score examination in total elderly subjects with diabetes ($n= 170$), and it is statistically significant $P < 0.001$ (Figure 3).

Table 1: Comparison of demographic data between the studied groups:

	Elderly without diabetes (n=85)		Elderly with controlled diabetes (n=85)		Elderly with Uncontrolled diabetes (n=85)		Test	P
Age (Years)							KW	0.673
Mean± SD	75.27 ± 7.14		74.61 ± 6.19		75.29 ± 5.77		0.791	(NS)
Median (Range)	73 (66 – 88)		74 (65 – 88)		74 (66 – 91)			
Sex							χ^2	0.822
Male	53	62.4%	51	60%	49	57.6%	0.392	(NS)
Female	32	37.6%	34	40%	36	42.4%		
Smoking Status							χ^2	0.720
Non-Smoker	58	68.2%	53	62.4%	55	64.7%	0.656	(NS)
Smoker	27	31.8%	32	37.6%	30	35.3%		
BMI							KW	0.17
Mean± SD	28.20 ± 2.26		27.83 ± 2.06		27.65 ± 2.12		3.54	(NS)
Median (Range)	27.5 (25 – 34)		27 (25 – 33)		27 (25 – 34)			

KW = Kruskal Wallis test χ^2 Chi-squared test. A p value < 0.05 was considered statistically significant (S).

Table 2: Comparison of MMS of the studied population ($n=255$):

	Elderly without diabetes (n=85)	Elderly with controlled diabetes (n=85)	Elderly with uncontrolled diabetes (n=85)	Test	P
MMS examination				F	<0.001 (S)
Mean± SD	26.41 ± 2.11	22.76 ± 2.89	18.41 ± 3.54	161.44	
Median (Range)	26 (22 – 30)	23 (18 – 28)	18 (13 – 25)		

F= One way ANOVA test. A p value <0.05 was considered statistically significant(S).

Table 3: Comparison of hs-CRP of the studied population (n=255):

	Elderly without diabetes (n=85)	Elderly with controlled diabetes (n=85)	Elderly with uncontrolled diabetes (n=85)	Test	P
Hs-CRP (mg/dL)				KW	<0.00001 (S)
Mean± SD	1 ± 0.9	2.4 ± 1.2	4.4 ± 1.6		
Median (Range)	0.8 (0 – 3.7)	2.3 (0.8 – 6.6)	4.6 (0.5 – 6.9)	146.11	

Table 4: LSD post hoc test of MMS between studied groups:

	Elderly with controlled diabetes (22.76 ± 2.89)	Elderly with uncontrolled diabetes (18.41 ± 3.54)
Elderly without diabetes (26.41 ± 2.11)	<0.001 (HS)	<0.001 (HS)
Elderly with controlled diabetes (22.76 ± 2.89)	---	<0.001 (HS)

Table 5: Multivariable linear regression analysis of factors affecting MMS

Variables	Beta	95% CI	P- value
IL-6	2.5	1.1 - 5.2	<0.05
hs-CRP	1.4	0.5 - 3.6	<0.05
TNF	1.1	0.1 - 4.7	>0.05

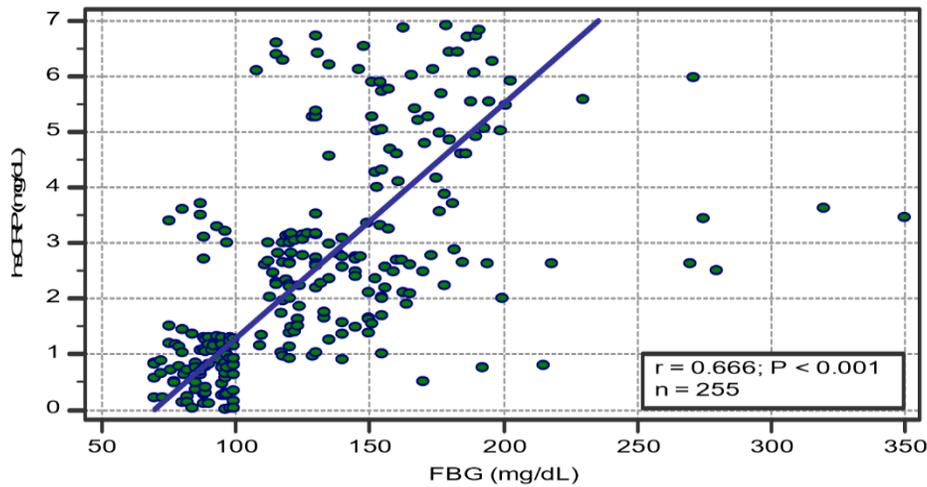


Figure 1: linear regression line between FBG (mg/dL) and hsCRP (mg/dl) in total study population there is statistically significant positive correlation between hs-CRP and FBG.

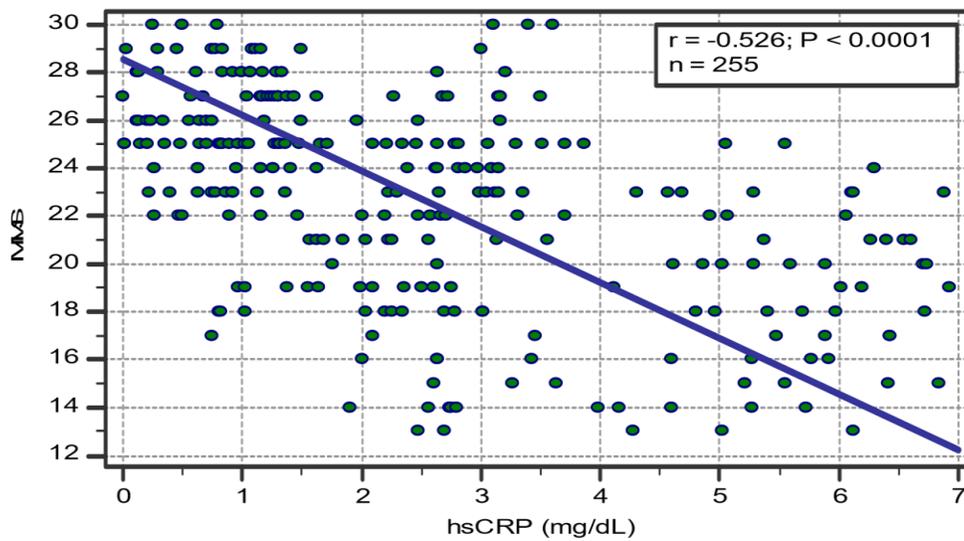


Figure 2: linear regression line between hsCRP (mg/dL) and MMS examination in total study population.

Elderly without diabetes

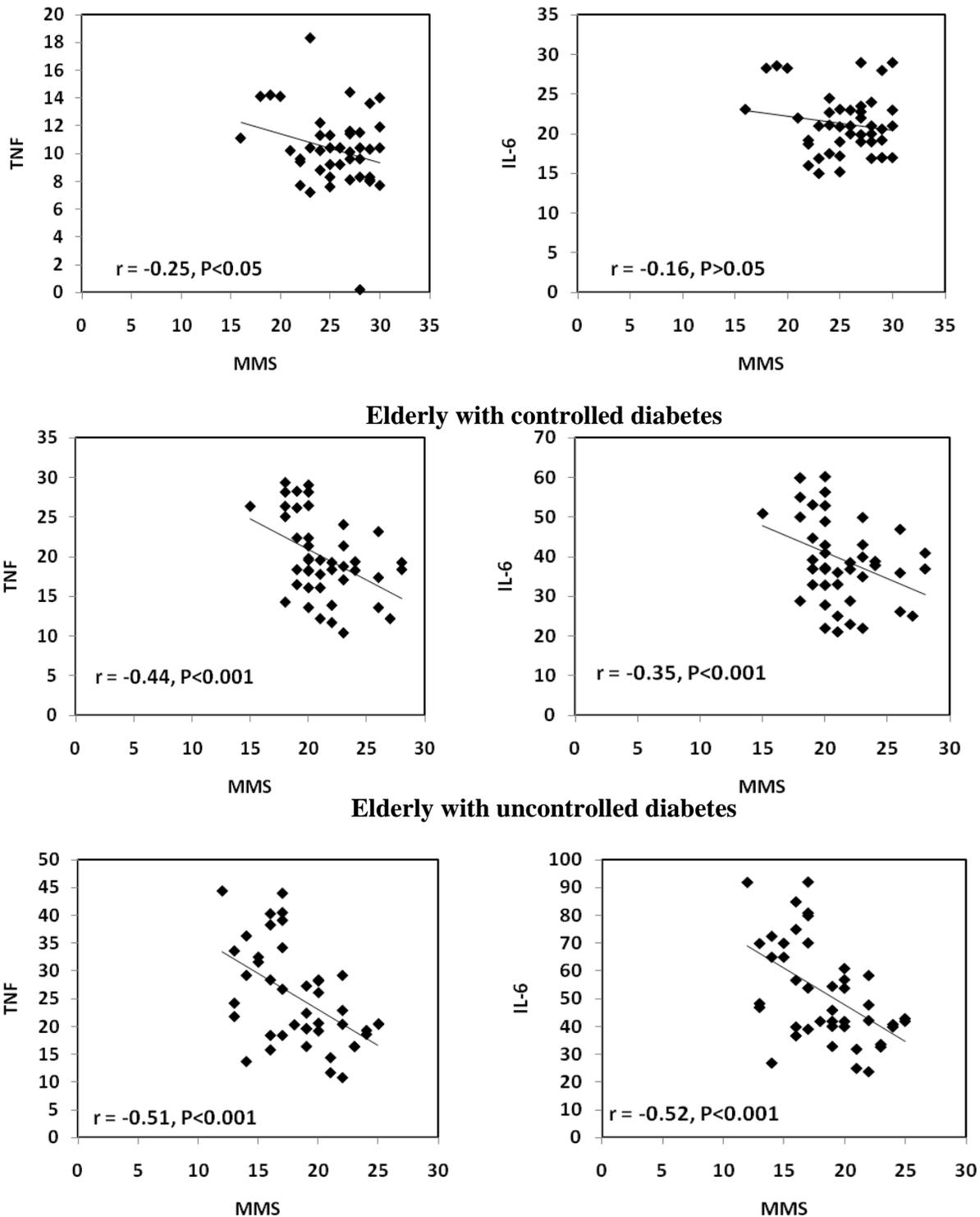


Figure 3: correlation between TNF- α , IL-6 and MMS in different group of the study.

DISCUSSION

Cognitive dysfunction is considered a significant issue and is increasing around the world, particularly among the elderly. For

instance, in a study expressed that the prevalence of dementia (the most serious type of clinically analyzed cognitive disorder) was assessed to be 8% for people above 65 years old

and 34% for those with age more than 85 years (10).

This study shows that hs-CRP, IL-6 and TNF- α are increased in elderly diabetics regardless of diabetic control compared with elderly without diabetes, this was in accordance with **Marioni *et al.*** (7) and **Gorska *et al.*** (11) who stated that hs-CRP levels in diabetic patients are known to be higher than in other subjects which may be due to status of chronic low-grade inflammation besides direct effects on cognition function, inflammation could also affect cerebral vaso-regulation. (7, 11), **Cernea & Dobreanu** stated that pathogenic mechanisms that linked to type 2 diabetes mellitus (T2DM) also include low-grade system inflammation processes (12).

It is clear that CRP will remain a predictor of development of diabetes independent of other clinically applicable predictors, as baseline BMI and fasting triglyceride and glucose levels as stated by **Freeman *et al.*** (13) Obviously, CRP can be considered as diabetes mellitus predictor in middle-aged males apart from other established risk factors and that degree of low-grade inflammation is critical in development of type 2 diabetes.

So many evidences suggest that inflammation may play an intermediary role in the mechanism of pathogenesis, thereby linking diabetes mellitus with many commonly coexisting health conditions thought to be originated via inflammatory pathways as described by **Festa *et al.*** (14). It is consistent with our study as we found that hs-CRP is significantly elevated in elderly individuals with diabetes regardless the level of diabetic control .

Diabetes is known as a risk factor for the cognitive dysfunction especially in elderly. Type 2 diabetes is associated with roughly a 2-fold rise in the risk of dementia compared to control (15).

In our study, we found that there was a difference between elderly with diabetes in contrast to those without diabetes as regard MMS examination which was statistically significant. Also, this difference was noted between those with controlled diabetes and those with uncontrolled diabetes. This was in agreement with **Biessels *et al.*** (16) who found that

high risk of dementia increases in patients with diabetes on the long run by a factor of two diabetes also increases the risk of progression of MCI to dementia. As well, **Ohara *et al.*** (17) demonstrated that the occurrence of all-causative factors of dementia, Alzheimer's disease (AD) and vascular dementia were altogether higher in patients with diabetes than in those with typical glucose resistance.

In this study there was statistically significant positive correlation between hs-CRP and FBG, A1C. In line with our findings, **Gorska *et al.*** (11) have described relationships between CRP, and glycosylated hemoglobin and also was significant. Patients with cognitive impairment had also more micro complications. This may be explained by sustained hyperglycemia which enhance development of diabetic complications can make chronic low-grade inflammation and cell activation via AGEs–RAGE interaction.

In current study there was statistically significant negative correlation between hs-CRP, IL-6, TNF- α and MMS score examination in whole population and was also significant in elderly with diabetes. **Sharma *et al.*** (18) have analyzed high-sensitivity c-reactive protein as inflammatory biomarker in type 2 diabetic mellitus and concluded that elevated level of hs-CRP in diabetic subjects was observed with insulin resistance and has strong cardio vascular risk marker in these individuals and that hs-CRP may be a marker of memory and visuospatial impairment in the elderly

As regarding IL-6 and TNF- α this was in accordance with **Zhao *et al.*** (19) who stated that higher circulating level of these inflammatory markers in diabetics are negatively correlated with cognitive function of those patients.

Another study by **Weinstien *et al.*** (20) studied the link of C-reactive protein (CRP) with subsequent cognitive abilities and decline among elderly individuals with cardiovascular disease (CVD) that was existing before, it was found that systemic chronic inflammation (CRP levels were positively related to a greater decline in executive functions)

In a study by Groska *et al.* ⁽¹¹⁾ they have found that the level of inflammatory markers – hsCRP was included in the study – could be considered as an additional criterion of long-term cognitive impairment and it was independent predictors of patients' cognitive status.

Limitations of the study: weak cooperation of some patients or their relatives was a point of difficulty for the study, also the expense of special laboratory investigations was very high.

In conclusion, it was found that there is statistically significant negative correlation between hs-CRP, IL-6, TNF- α and MMS score examination in total elderly subjects with diabetes. As well, there is significant positive correlation between hs-CRP and parameters of diabetic control. so inflammatory markers in elderly patients with diabetes may be a reliable indicator to identify and anticipate cognitive dysfunction in such individuals.

Recommendations: we hope that the coming studies will include insulin dependent group either type 1 or type 2DM to correlate cognitive function with different inflammatory markers.

Conflict of interest:

The authors declared that they have no conflicts of interest with respect to the authorship and/ or publication of this article.

Financial Disclosures

This study was not supported by any source of finding.

Acknowledgement

The authors would like to appreciate all the participants and the hospital staff who contributed to this study.

Author contributions

All the authors carried out this work. **Amira Ahmed Mahmoud** and **Nancy Abdelhamid Mohammad** designed the study and collected the patients, **Ahmad Sallam Soliman** did the laboratory works. All the authors conduct analysis and interpretation of the data and write the manuscript. All authors were involved in drafting the article and revising it for important intellectual content and all authors read and approved the final version to be published.

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Mahmoud, A., Mohammad, N., Soliman, A. THE RELATIONSHIP BETWEEN HIGH SENSITIVE C- REACTIVE PROTEIN, INTERLEUKIN-6 , TUMOR NECROSIS FACTORS - α AND COGNITIVE FUNCTION IN ELDERLY DIABETICS IN ZAGAZIG UNIVERSITY HOSPITALS. *Zagazig University Medical Journal*, 2022; (333-341): -. doi: 10.21608/zumj.2021.107835.2419