Al-Turath Medical Journal (ATMJ)
ISSN Print: 3080-1265
Online : 3080-1273

Exploring IL_18 Levels in Alzheimer's disease progression Using ELISA Methodology

Sanan Th., Imad Sh. and Kismat T.

Department of Medical Laboratories Techniques, Al-Turath University, Baghdad, Iraq.
Correspondence email: **sanan.thaer@uoturath.edu.iq**

KEYWORDS
IL_18, Alzheimer's disease, ELISA

Received: 05/10/2024
Accepted: 22/12/2024
Available online 31/12/2024

ABSTRACT
The Alzheimer disease (AD) is characterized by cognitive deterioration and complex pathophysiology, which comprises neuroinflammation. Interleukin-18 (IL-18) is a proinflammatory cytokine associated with the development of AD with high concentrations of the protein observed in patients. The involvement of IL-18 in neuroinflammation, the increase of the cytokine in response to microglial activation, as well as its action on other immune cells, are also pathological features of AD.
The current research investigates how the level of Interleukin-18 (IL-18) plays a role in the development of Alzheimer Disease (AD) by the Enzyme-Linked Immunosorbent Assay (ELISA) analysis. It explores the intricate pathophysiology of AD, noting how important neuroinflammation is in the disease and the role of IL-18 as a proinflammatory cytokine in its development. The research used ELISA in quantifying the IL-18 levels in 20 patient samples and 20 healthy control samples offering evidence on the relationship between IL-18 expression and severity of AD. The outcomes showed that there was a significant increase in IL-18 in patients with AD (59.38) compared to the controls (14.38), thus highlighting the potential use of IL-18 as a biomarker of AD development and participation of IL-18 in the inflammatory mechanisms of the disease. These results indicate that IL-18 may represent an interesting therapeutic target leading to a new way of managing the AD and its treatment

DOI : https://doi.org/10.63964/atmj.2024.1.2

1-INTRODUCTION

Most prevalent type of dementia among aged is the neurodegenerative disorder, alzheimer disease (AD) that exhibits cognitive impairment and progression. The pathology of AD is complex meaning that there are numerous molecular and cellular pathways involved in the loss of and the dysfunction of the neurons. In biomolecular aspects, inflammation is another one of the phenomena that has been identified as an essential player in the progression of AD (1). Interleukin-18 (IL-18) is one of the proinflammatory cytokines whose importance in the process of inflammation and the pathogenesis of a number of chronic inflammatory diseases is near-continually being recognized. Within the framework of AD IL-18 is of high interest because of the possibility of having direct influence on the long-term neuroinflammation processes in the brain tissue of AD patients. Higher concentration of IL-18 was observed in the cerebrospinal fluid and brains tissues after mortem of people

with AD, which seems to indicate dependence of IL-18 expression on the level of the disease (2).

Brain inflammation is one of the characteristic features of AD, which is manifested by the activation of microglia and the following production of proinflammatory factors. Activated microglia have been found to produce the IL-18, which has been reported to trigger the synthesis of other cytokines of the inflammatory cycle, increase the expression of adhesion molecules on endothelial cells, and regulate the functioning of natural killer cells and T lymphocytes. Chronic inflammation of AD brain is associated with abundant production of IL-18 that aggravates the neural injuries and facilitates the degenerative process (3).Enzyme-Linked Immunosorbent Assay (ELISA) is a very sensitive analytical biochemistry technique which has been used to determine levels of a substance (usual proteins like cytokines) in a biological fluid using antibodies. Its use is preferable because of specificity, sensitivity and the versatility of ELISA applied in multiple research and clinical environments. ELISA has been used in the study of AD in order to ascertain the levels of IL-18 in samples taken by patients in order to provide a guiding factor in the interdependence between the level of IL-18 expression and the progression of AD (4). The analysis of the IL-18 levels by ELISA technology available enables the analysis of the cytokine patterns in various phases of AD as well as offers the opportunity to investigate the prospect of IL-18 as a biomarker in the identification and progression of AD. Moreover, high throughput nature of ELISA means that it would be suitable method to carry out a large-scale experiment to examine the dynamics involved in the expression of IL-18 with other inflammatory markers in AD (5). Dissolving the function of IL-18 in the AD can be an important treatment implication. Provided that the level of IL-18 is correlated with the development of disease, this protein can be used as a target of drug treatment. Discovery of IL-18 inhibitors has a potential to alleviate the inflammation processes implicated in AD, providing new opportunity in the treatment. Also, the determination of IL-18 level by ELISA can serve as a good aid in tracking the impact of medical intervention in modulating neuroinflammatory reactions in AD (6).
In the course of research, it will be essential to explain the pathways through which IL-18 builds up AD pathology and the potential of IL-18 as a target of intervention or a diagnostic indicator. ELISA methodology in the study of AD therefore can be considered a fruitful source of further investigation of the disease and of the evolution of new tactics of struggle with this nuisance. Objective of this study to learnthe role of IL-18 in AD it will be imperative to elucidate the avenues through which the IL-18 plays a role in pathology of AD and to clarify the value of IL-18 as a therapeutic possibility, or a biomarker.

Materials and methods

Apparatus

The instruments used in the present study are listed with the producing company and the country in table (3-1).

Table (3-1): Instruments used in this study.

No.	Instruments	Origin
1	Centrifuge	Germany
2	Deep Freezer (-20 ^{0}C)	Iraq
4	Rotatory Shaker	England
5	Spectrophotometer	Germany
6	Vortex mixer	Tunisia
7	Automatic micro-pipettes	USA
8	Dri-Chem NX500	Japan
10	Automatic multi-channel pipettes	
11	Beakers	
12	Cotton balls	
13	Cylinder	
14	Disposable gloves	
15	Disposable plain tubes	
16	Disposable syringes	
17	Biopette Variable Volume (2-20 20-200,100-1000) µl	Eppendorf
19	Tips (blue, yellow)	AFCO, Jordan
20	Eppendorf tube (1.5µl)	Abod, Korea
21	Incubator	Chain
22	Deep Freezer	Sanyo/ Japan
24	Distillator	china
25	Eppindorf tube (1.5 ml)	China
26	Spectrophotometer	china

Materials

The materialsused in the present study are listed with the producing company and the country in table (3-2).

Table (3-2): materials used in this study.

Kit	Company/ origin
IL-18 ELISA Kit	CUSABIO/ China
Alcohol (70%)	-
Distilled water	-
Drabkin's solution	-

2-METHODS

Samples collection

The investigation encompassed 20 individuals undergoing evaluation for Alzheimers at a specialized medical facility. Additionally, 20 healthy samples were also included in this study as a control group.

Study design.

The steps that included in this study can be summarized in the figure (3-1).

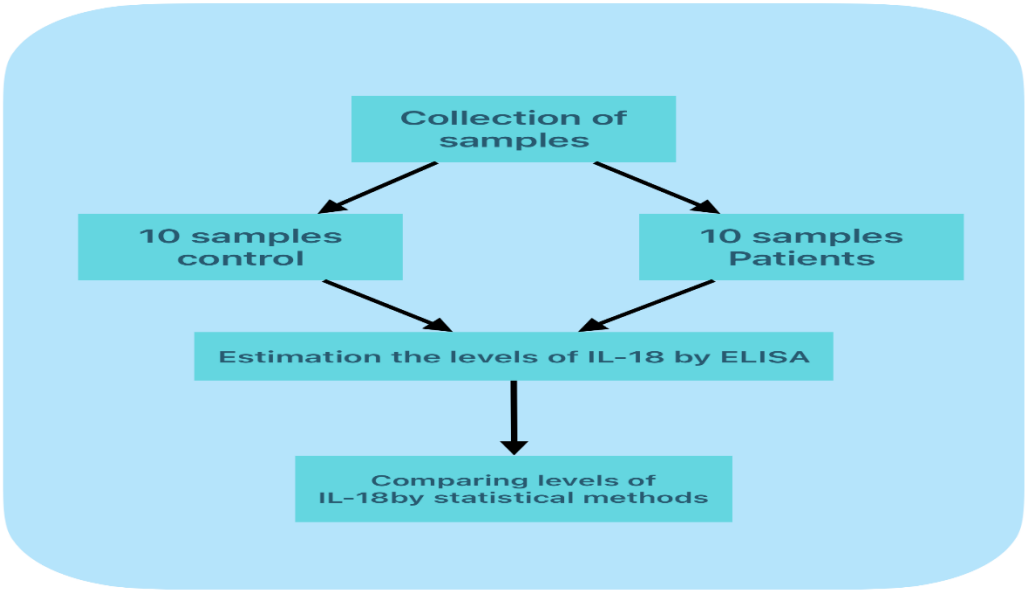

Figure (3-1); study design steps

Estimation the level of IL-18

A) AssayPrinciple

This assay kit operates on the principle of Enzyme-Linked Immunosorbent Assay (ELISA) and is specifically designed for detecting Human IL-18. The assay involves a plate pre-coated with antibodies against Human IL-18. When a sample containing IL-18 is introduced, it attaches to these antibodies. Following this, a biotinylated antibody targeting Human IL-18 is added, which binds to the IL-18 in the sample. Subsequently, Streptavidin-HRP is introduced, binding to the biotinylated IL-18 antibody. Post-incubation, any unattached Streptavidin-HRP is removed during the washing process. The addition of a substrate solution leads to a color change proportional to the Human IL-18 concentration in the sample. The process concludes with the application of an acidic stop solution, and the absorbance is measured at 450 nm to quantify the IL-18 present.

B) ReagentPreparation

Prior to use, all reagents are required to be stabilized to room temperature. The standard, consisting of 120µl at a concentration of 640ng/L, is reconstituted with an equal volume of standard diluent, resulting in a 320ng/L standard stock solution. This solution is then allowed to equilibrate for 15 minutes with gentle agitation before dilution. Duplicate standard points are produced by serial dilution of the standard stock solution (320ng/L), diluted 1:2 with standard diluent to create solutions of 160ng/L, 80ng/L, 40ng/L, and 20ng/L. The zero standard (0 ng/ml) is established using the standard diluent alone. Remaining solutions are preserved by freezing at -20°C and are recommended to be used within one month. The standard solutions are diluted as suggested in the protocol provided.

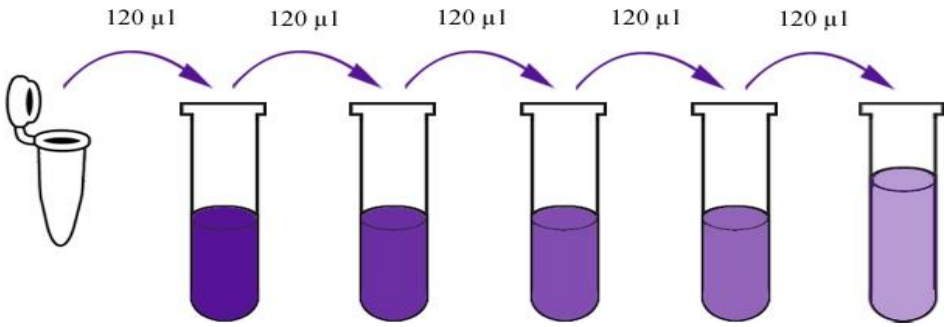

Figure 3-2 Dilutions preparation

Table 3-3 Original Standard and Standard Diluent

Table 3-4 Standards

Standard Concentration	StandardNo.5	StandardNo.4	StandardNo.3	StandardNo.2	StandardNo.1
320ng/L	StandardNo.5	120µlOriginalStandard+ 120µlStandardDiluent			
160ng/L	StandardNo.4	120µlStandard No.5+ 120µlStandardDiluent			
640ng/L 80ng/L	320ng/L StandardNo.3	160ng/L 120µlStandard No.4+ 120µlStandardDiluent	80ng/L	40ng/L	20ng/L
40ng/L	StandardNo.2	120µlStandard No.3+ 120µlStandardDiluent			
20ng/L	StandardNo.1	120µlStandard No.2+ 120µlStandardDiluent			

The preparation of the Wash Buffer is completed by diluting 20ml of the 25x Wash Buffer Concentrate into deionized or distilled water, resulting in a total of 500 ml of 1x Wash Buffer. Should there be any crystallization within the concentrate, it is resolved by gentle stirring until the crystals have fully dissolved.

C) Procedures

1. Reagents, standard solutions, and samples were prepared as specified and brought to room temperature prior to use. The entire assay procedure was carried out at room temperature.
2. The required number of strips for the assay was determined, and these strips were placed into frames for usage. Any strips not used were stored at temperatures between 2-8¬∞C.
3. To the designated standard well, 50Œºl of standard was dispensed. It is important to note that antibody was not added to the standard well due to the presence of biotinylated antibody within the standard solution itself.
4. For the sample wells, 40Œºl of sample was introduced, followed by the addition of 10Œºl of anti-IL-18 antibody. Subsequently, 50Œºl of streptavidin-HRP was added to both

the sample and standard wells, with the exception of the blank control well. Following thorough mixing, the plate was sealed and incubated for 60 minutes at a temperature of 37¬∞C.

5. After incubation, the sealer was removed, and the plate was subjected to a washing process five times using the wash buffer. The wells were filled with at least 0.35 ml of wash buffer for a duration ranging from 30 seconds to a minute for each wash. In the case of automatic washing, the contents of each well were aspirated or decanted, followed by five washes with the wash buffer. Excess buffer was then removed by blotting the plate onto paper towels or other absorbent materials.
6. Each well then received 50Œºl of substrate solution A, succeeded by 50Œºl of substrate solution B. The plate was covered with a new sealer and incubated for 10 minutes at 37¬∞C in the absence of light.
7. Stop Solution of 50Œºl was introduced to each well, leading to an immediate color change from blue to yellow.
8. The optical density, also known as the OD value, of each well was assessed promptly using a microplate reader calibrated to 450 nm. This measurement was performed within 10 minutes of adding the stop solution.

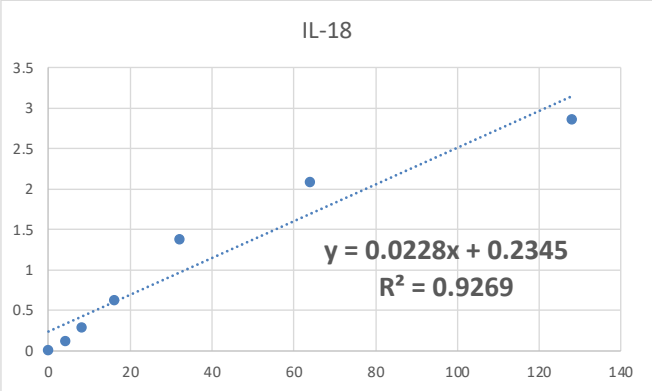

Figure 3-3 Standard Curve.

Results

In the studied cohort, the control (20 samples) group has an average age of 58.42 years with a standard error of 9.37, whereas the patients (20 samples) diagnosed with Alzheimer's disease are older, with an average age of 73.23 years and a standard error of 12.34. Despite the apparent age difference between the two groups, the p-value of 0.902 indicates that this age disparity is not statistically significant (NS), suggesting that within this sample, age alone may not be a distinguishing factor between controls and those with Alzheimer's. Conversely, when examining the family history of Alzheimer's disease, none of the participants in the control group reported a family history of the condition (0%), while 30% of the patients with Alzheimer's had a positive family history. The p-value for this comparison is highly significant (0.0001**), indicating that the difference observed between the control and Alzheimer's groups in terms of family history is statistically significant. This suggests a potential genetic or hereditary component in the prevalence of Alzheimer's disease within the study population. The significant p-value associated with family history underscores the potential importance of genetic factors in Alzheimer's disease risk. Conversely, the nonsignificant p-

value related to age differences between the groups suggests that while age is a known risk factor for Alzheimer's disease, in this particular sample, it was not significantly different between the control group and those with the condition.

Table Error! No text of specified style in document.**-1; Comparative Analysis of age and family history Mean and Standard Error between Alzheimer's Patients and Controls**

Characteristics		**Control group**	**Patients' Alzheimer's**	***P* value**
Age		58.42 ±9.37	73.23 ±12.34	0.902 (NS)
Family history	yes	0 (0%)	6(30%)	**0.0001 ****
	no	20 (100%)	14(70%)	

Figure 4.1 illustrates a comparative analysis of the mean interleukin-18 (IL-18) levels between patients diagnosed with Alzheimer's disease and a control group. The analysis reveals that patients with Alzheimer's disease exhibit a mean IL-18 concentration of 59.38 units, which is significantly higher than the mean value of 14.83 units reported in the control group. This marked difference in IL-18 levels suggests a strong association between the elevated presence of this cytokine and the pathological state of Alzheimer's disease. The data, derived from the Enzyme-Linked Immunosorbent Assay (ELISA) methodology, underscore the potential role of IL-18 as a biomarker for the progression of Alzheimer's disease, reflecting the heightened inflammatory state that may be inherent to the disease's progression. The findings warrant further investigation into the mechanistic link between IL-18 elevation and Alzheimer's disease and suggest that IL-18 may be a valuable target for therapeutic intervention and disease monitoring

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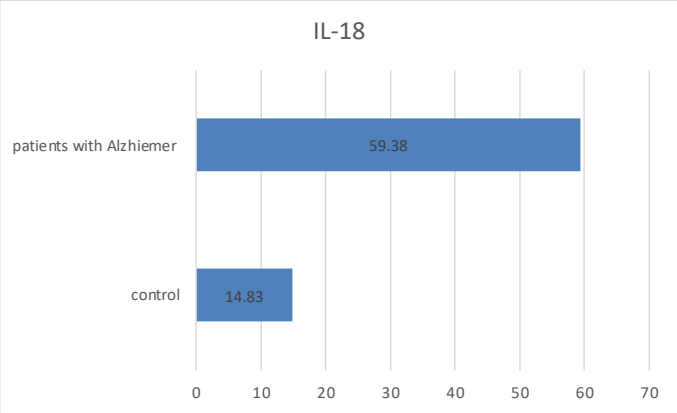

FigureError! No text of specified style in document.**-1; Comparative Analysis of Mean between Patients and Controls**

4-DISCUSSION

With regard to Alzheimer disease inflammation is emerging as an important factor which can be a contributory mechanism of disease. As the findings of the study reveal, as shown in Figure 4.1,

there is a great evidence supporting the effects of the relationship between high levels of interleukin-18 (IL-18) and AD. It is indicated by the data that IL-18 concentrations in patients with AD are significantly higher than in a control sample, and IL-18 may play a role in the neuroinflammation process which was the characteristic feature of AD (7).

The IL-18 is a pro-inflammatory cytokine, which is central in immune response. Its elevated concentrations among AD patients corroborate with the assumptions that inflammatory pathways are stimulated in the neurodegenerative disorders. It is especially interesting how the average level of IL-18 in AD patients is four times higher than in the control group, which proves the idea that IL-18 is not merely a spectator but may effectively be engaged in the development of the disease (8).

Although the exact mechanisms, through which IL-18 could have a role in AD, are not yet comprehensively covered, it is possible to propose several mechanisms through which it could play a role. IL-18 can worsen neuronal injury by stimulating brain inflammatory cells or also voicing an increase in the generation of other inflammatory components, which may cause a dysfunction in synapses and neural death. Alternatively, IL-18 would affect the processing or deposit of the amyloid-beta, a pathological hallmark of AD (9).

This study has employed the ELISA technique of analysis, which provides a sensitive and solid method of determining and quantifying the IL-18 activity, which confirms the validity of the research conclusions.

The high specificity of the methodology ensures that it is a very valuable procedure in the identification of a biomarker and here it has helped in the unraveling of a prospective biomarker in AD development (10).

Considering these findings, IL-18 can be viewed as a possible specimen of future studies regarding its use as an early detection marker, disease monitoring and even prognosis of AD. In addition, in case IL-18 is involved in pathogenic pathways in AD, it can be used as an object of treatment. Dampening of IL-18 levels or inhibiting its action may have a prospective effect on modifying the direction of AD, which offers a new direction of treatment (11).

But it is necessary to remember the fact that correlation does not equal causation. Hence, although high IL-18 levels are linked to AD, it is yet to be identified through further research whether IL-18 has a causative effect on AD. IL-18 longitudinal studies and/or interventional studies in which IL-18 was manipulated will go a long way in determining the role of IL-18 in AD (12).

Conclusion

To sum up, the results of the study indicating the increase in IL-18 in AD patients raise new possibilities of the research around inflammation as the factor in AD and offer new possibilities of creating diagnostic and management methods. It is vital that further studies base on such results to develop further on the potentials of IL-18 as biomarker and therapeutic target in Alzheimer disease.

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