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

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

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KEYWORDS
IL_18, Alzheimer's disease, ELISA

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

ABSTRACT
Alzheimer's disease (AD) is marked by cognitive decline and involves complex pathophysiology, including neuroinflammation. Interleukin-18 (IL-18), a proinflammatory cytokine, is linked to AD's progression, with elevated levels found in patients. IL-18's role in neuroinflammation, including its production by microglia and effects on other immune cells, contributes to AD's pathology.
This study explores the role of Interleukin-18 (IL-18) levels in the progression of Alzheimer's Disease (AD) using the Enzyme-Linked Immunosorbent Assay (ELISA) methodology. It investigates the complex pathophysiology of AD, highlighting the significance of neuroinflammation and the contribution of IL-18 as a proinflammatory cytokine in the disease's progression. The study employed ELISA to quantitatively measure IL-18 levels in 20 patient samples and 20 healthy control samples, providing insights into the correlation between IL-18 expression and AD severity. Results revealed a marked elevation of IL-18 in AD patients (59.38) compared to controls (14.38), underscoring the cytokine's potential role as a biomarker for AD progression and its involvement in the disease's inflammatory processes. These findings suggest that IL-18 could be a target for therapeutic intervention, offering new avenues for AD management and treatment

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

1-INTRODUCTION

Alzheimer's disease (AD), a neurodegenerative disorder characterized by progressive cognitive decline, is the most common cause of dementia in the elderly population. The pathophysiology of AD is complex, involving multiple molecular and cellular pathways that contribute to neuronal loss and dysfunction. Among the various biomolecular changes associated with AD, inflammation has been recognized as a significant factor in the disease's progression (Scheltens *et al*., 2021). Interleukin-18 (IL-18) is a proinflammatory cytokine that plays a critical role in the inflammatory response and has been implicated in the pathogenesis of several chronic inflammatory diseases. In the context of AD, IL-18 is of particular interest due to its potential involvement in the chronic neuroinflammatory processes observed in the brains of AD patients. Elevated levels of IL-18 have been reported in the cerebrospinal fluid and post-mortem brain tissues of individuals with AD, suggesting a correlation between IL-18 expression and the severity of the disease (Griffin *et al*., 2006).

Neuroinflammation is a hallmark of AD, characterized by the activation of microglial cells and the subsequent release of inflammatory mediators. IL-18 is known to be produced by activated microglia and has been shown to induce the production of other inflammatory cytokines, enhance the expression of adhesion molecules on endothelial cells, and modulate the activity of natural killer cells and T lymphocytes. The overproduction of IL-18 in the AD brain may contribute to the chronic inflammatory state, exacerbating neuronal damage and promoting the progression of the disease (Ojala *et al*., 2009).

The Enzyme-Linked Immunosorbent Assay (ELISA) is a powerful analytical biochemistry assay that utilizes antibodies to measure the concentration of a substance, typically proteins such as cytokines, in a biological fluid. ELISA is favored for its specificity, sensitivity, and versatility in various research and clinical settings. In the study of AD, ELISA has been utilized to quantify IL-18 levels in patient samples, offering insights into the relationship between IL-18 expression and AD progression (Dinarello, 1999). The quantification of IL-18 using ELISA methodology allows for the examination of cytokine profiles in different stages of AD and provides a means to explore the potential of IL-18 as a biomarker for AD diagnosis and progression. Furthermore, ELISA's high-throughput capability makes it an ideal method for large-scale studies aimed at understanding the dynamics of IL-18 expression in relation to other inflammatory markers in AD (Tan *et al*., 2007). Understanding the role of IL-18 in AD could have significant therapeutic implications. If IL-18 levels correlate with disease progression, they may serve as a target for therapeutic intervention. The development of IL-18 inhibitors could potentially mitigate the inflammatory processes involved in AD, offering a novel approach to treatment. Additionally, IL-18 levels measured by ELISA may provide a valuable tool for monitoring the efficacy of therapeutic interventions aimed at modulating neuroinflammatory responses in AD (Bossù *et al*., 2010).

As research progresses, it will be critical to elucidate the mechanisms by which IL-18 contributes to AD pathology and to clarify the potential of IL-18 as a therapeutic target or a biomarker. The application of ELISA methodology in AD research thus represents a promising avenue for advancing our understanding of the disease and for the development of new strategies to combat this debilitating condition. Aim of this study to uunderstanding the role of IL-18 in AD it will be critical to elucidate the mechanisms by which IL-18 contributes to AD pathology and to clarify the potential of IL-18 as a therapeutic target 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).

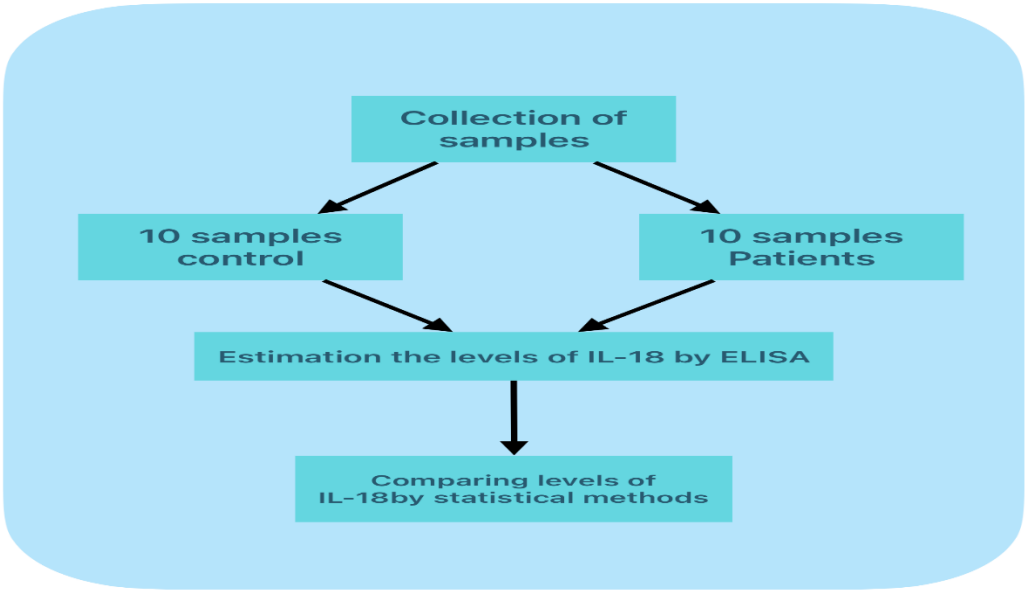

Figure (3-1); study design steps

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.

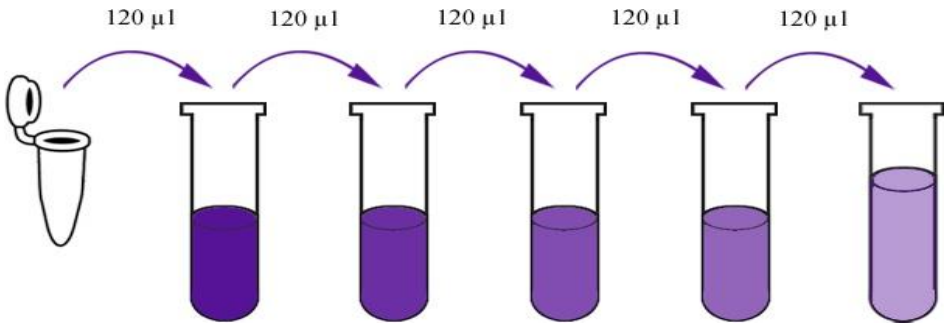

Figure 3-2 Dilutions preparation

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.

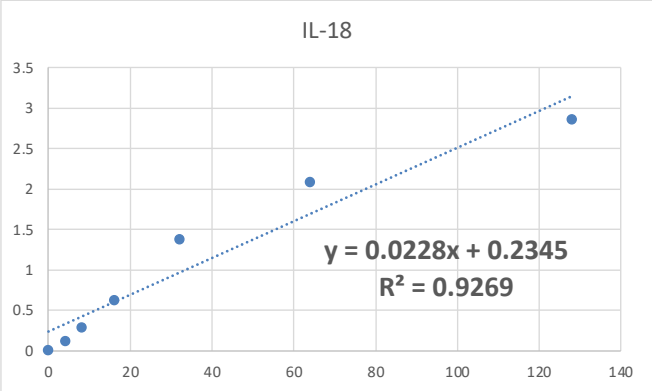

Figure 3-3 Standard Curve.

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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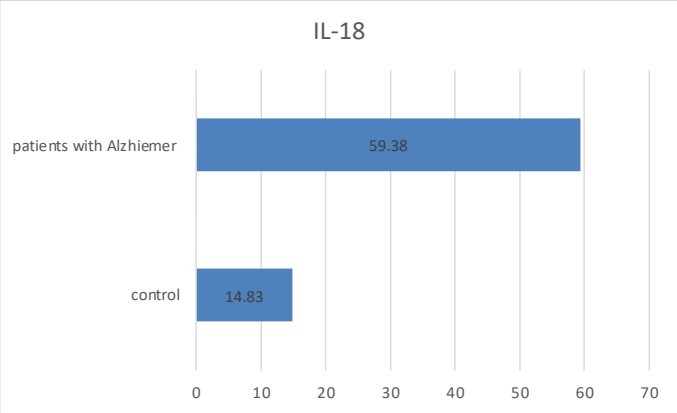

FigureError! No text of specified style in document.**-1; Comparative Analysis of Mean between Patients and Controls**

4-DISCUSSION

In the context of Alzheimer's disease inflammation is increasingly recognized as a significant factor that may contribute to disease pathogenesis. The study's findings, as presented in Figure 4.1,

provide substantial evidence for the association between elevated interleukin-18 (IL-18) levels and AD. The data show that IL-18 concentrations are significantly higher in patients with AD compared to those in a control group, suggesting that IL-18 could be involved in the neuroinflammatory processes that characterize AD (Kinney *et al*., 2018).

IL-18 is a pro-inflammatory cytokine that plays a central role in the immune response. Its increased levels in AD patients align with the hypothesis that inflammatory pathways are activated in neurodegenerative disorders. The fact that the mean IL-18 levels in AD patients are four times higher than in the control group is particularly striking and supports the notion that IL-18 is not merely a bystander but could be actively involved in the disease's progression (Fabbi *et al*., 2015).

While the exact mechanisms by which IL-18 might contribute to AD are not yet fully understood, several possible pathways can be proposed. IL-18 may exacerbate neuronal damage through the activation of inflammatory cells in the brain or by promoting the production of other inflammatory mediators that can lead to synaptic dysfunction and neuronal death. Alternatively, IL-18 might influence the processing or accumulation of amyloid-beta, a hallmark of AD pathology (Landy *et al*., 2024).

The use of the ELISA method in this study offers a robust and sensitive approach to detect and quantify IL-18 levels, reinforcing the credibility of the findings.

The methodology's precision makes it an invaluable tool in biomarker discovery, and in this case, it has facilitated the identification of a potential biomarker for AD progression (Alsaleh *et al*., 2009).

Given these results, IL-18 represents a promising candidate for further research into its role as a biomarker for early detection, disease monitoring, and even prognosis of AD. Moreover, if IL-18 is implicated in the pathogenic pathways of AD, it may also serve as a target for therapeutic interventions. Modulating IL-18 levels or blocking its activity could potentially alter the course of AD, providing a novel approach to treatment (Landy *et al*., 2024).

However, it is important to note that correlation does not imply causation. Therefore, while elevated IL-18 levels are associated with AD, further studies are needed to determine whether IL-18 plays a causal role in the disease. Longitudinal studies tracking IL-18 levels over time in relation to disease progression, as well as interventional studies modulating IL-18 activity, will be crucial to elucidate its role in AD (K *et al*., 2004).

Conclusion

In conclusion, the elevation of IL-18 in AD patients observed in this study opens new avenues for understanding the role of inflammation in AD and presents new opportunities for the development of diagnostic and therapeutic strategies. It is imperative that future research builds on these findings to fully unravel the potential of IL-18 as a biomarker and therapeutic target in Alzheimer's disease.

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