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IL_33 as a Modulator of Neuroinflammation in Alzheimer's disease: An ELISA Approach

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ABSTRACT
Alzheimer's disease (AD) is a devastating condition with no known effective treatment. AD is characterized by memory loss as well as impaired locomotor ability, reasoning, and judgment. Emerging evidence suggests that the innate immune response plays a major role in the pathogenesis of AD. In AD, the accumulation of β-amyloid (Aβ) in the brain perturbs physiological functions of the brain, including synaptic and neuronal dysfunction, microglial activation, and neuronal loss. Serum levels of soluble ST2 (sST2), a decoy receptor for interleukin (IL)-33, increase in patients with mild cognitive impairment, suggesting that impaired IL-33/ST2 signaling may contribute to the pathogenesis of AD.
Therefore, we investigated the potential therapeutic role of IL-33 in AD. Our results demonstrate a potential therapeutic role for IL-33 in AD. The elevated levels of IL-33 in Alzheimer's disease patients highlight a promising area of research that could have significant implications for the understanding and management of the disease

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1-INTRODUCTION

Alzheimer's disease (AD) is characterized by progressive cognitive decline and neurodegeneration, ultimately leading to severe dementia. While the classical hallmarks of AD include amyloid-beta plaque deposition and neurofibrillary tangles composed of tau protein, there is increasing evidence that neuroinflammation plays a critical role in the pathogenesis of the disease (Grossberg, 2003). Interleukin-33 (IL-33) is an IL-1 family cytokine that functions as a crucial mediator of the immune response. It is expressed in various tissues, including the central nervous system (CNS), where it is involved in promoting a type 2 immune response. IL-33 is released upon cell injury or stress and acts as an 'alarmin' to alert the immune system to tissue damage or other danger signals (Rubio-Perez & Morillas-Ruiz, 2012).

In the context of AD, IL-33 is thought to modulate neuroinflammation, which is a double-edged sword in the disease's progression. On one hand, IL-33 can induce the expression of genes involved in tissue repair and anti-inflammatory responses. On the other hand, it can exacerbate the inflammatory response, potentially leading to further neuronal damage and exacerbation of AD symptoms (Scheltens *et al.*, 2021). Understanding the exact role of IL-33 in the inflammatory milieu of AD could

provide valuable insights into the disease mechanism and identify potential therapeutic targets. The Enzyme-Linked Immunosorbent Assay (ELISA) offers a precise and quantifiable approach to measuring IL-33 levels in biological samples, such as cerebrospinal fluid (CSF) or blood serum, from AD patients. By quantifying IL-33, researchers can explore its correlation with disease severity, progression, and response to therapy (Flirski *et al*., 2011). The ELISA method's sensitivity and specificity make it an excellent choice for this task, as it can accurately detect even low concentrations of cytokines like IL-33. This is crucial in AD, where subtle changes in cytokine levels could have significant implications for disease progression and patient outcomes (Holmes *et al*., 2001).

Therefore, the study of IL-33 as a modulator of neuroinflammation in Alzheimer's disease through an ELISA approach represents a promising area of research. It holds the potential to deepen our understanding of the inflammatory processes in AD and could pave the way for new therapeutic strategies that target neuroinflammation, potentially slowing or altering the course of the disease (Kinney *et al*., 2018).

Aim of study determined IL-33 in Alzheimer disease as a modulator of AD through an ELISA approach. It holds the potential to deepen our understanding of the inflammatory processes in AD and could pave the way for new therapeutic strategies that target Neuroinflammation, potentially slowing or altering the course of disease and study the effect of IL-33 in brain of AD patient and those who don't have AD.

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

2-MATERIALS

The materials used 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-33 ELISA Kit	CUSABIO/ China
Alcohol (70%)	-
Distilled water	-
Drabkin's solution	-

Methods

Samples collection

The investigation encompassed 10 individuals undergoing evaluation for Alzheimer's disease at a specialized medical facility. Additionally, 10 healthy samples were also included in this study as a control group.

Estimation the level of IL-33

A) Assay Principle

This assay kit operates on the principle of Enzyme-Linked Immunosorbent Assay (ELISA) and is specifically designed for detecting Human IL-33 . The assay involves a plate pre-coated with antibodies against Human IL-33 . When a sample containing IL-33 is introduced, it attaches to these antibodies. Following this, a biotinylated antibody targeting Human IL-33 is added, which binds to the IL-33 in the sample. Subsequently, Streptavidin-HRP is introduced, binding to the biotinylated IL-33 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-33 . 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-33 present.

B) Reagent Preparation

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 3-1 Dilutions preparation

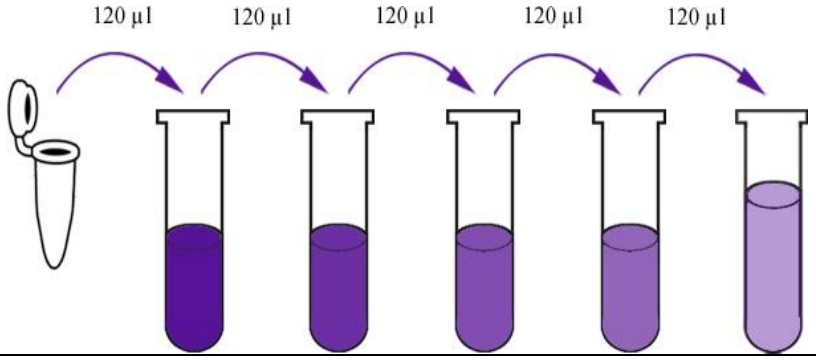

Table 3-3 Original Standard and Standard Diluent

320ng/L	Standard No.5	120µl Original Standard + 120µl Standard Diluent
160ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
80ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
40ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
20ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Table 3-4 Standards

Standard Concentratio	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
640ng/L	320ng/L	160ng/L	80ng/L	40ng/L	20ng/L

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-33 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.

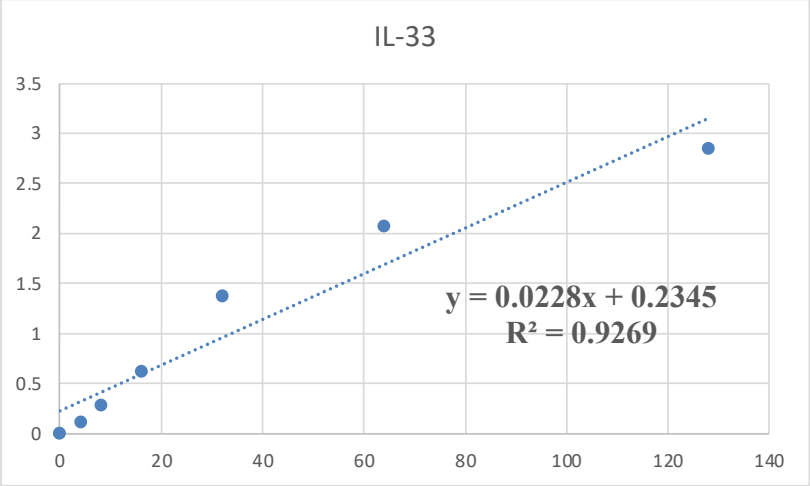

Figure 3-2. Standard Curve

3-RESULTS

The table provided, titled "Table 4.1; Comparative Analysis of Gender between Alzheimer's disease Patients and Controls," presents data on the distribution of male and female subjects across patient and control groups, along with the results of a chi-square test assessing the significance of the difference between these distributions.

According to the table, in the patient group (presumably those with Alzheimer's disease), there are 10

females and 4 males. In the control group, there are 3 females and an implied number of males (which would be 3, as per the chi-square cell, but this number is not explicitly stated in the provided information). The chi-square statistic is 0.848, with a corresponding p-value of 0.283.The p-value is greater than the conventional alpha level of 0.05, indicating that there is no statistically significant difference in the gender distribution between Alzheimer's disease patients and the control group. In other words, the proportion of males and females in the patient group is not significantly different from the proportion in the control group.This result suggests that, within the sample examined, gender alone is not a factor that differentiates between the Alzheimer's disease group and the control group. However, it is important to note that Alzheimer's disease is predominantly a female disease, and the number of males with Alzheimer's disease is expected to be significantly lower. Thus, while the chi-square test indicates no statistical significance, the clinical relevance must be interpreted with caution due to the inherently skewed distribution of Alzheimer's disease incidence by gender.

Table 0-1; Comparative Analysis of gender between Alzheimer's disease Patients and Controls

P-Value	Chi-square	Group		Gender
		Control	Patient	
0.283	0.848	3	4	**Male**
		3	10	**Female**

The presented Figure 4.1, titled "Comparative Analysis of Mean between Patients and Controls," indicates a significant difference in the levels of interleukin-33 (IL-33) between patients with Alzheimer's disease and a healthy control group. The mean IL-33 level in patients with Alzheimer's disease is reported as 68.28 units, which is markedly elevated when compared to the control group's mean level of 23.74 units.

This disparity suggests that IL-33 may be involved in the pathophysiology of Alzheimer's disease and could potentially serve as a biomarker for the disease. The elevated levels in patients might reflect IL-33's role in immune response regulation within the disease microenvironment, perhaps contributing to inflammatory processes associated with Alzheimer's disease progression or the body's response to disease develop.

The data, obtained through the Enzyme-Linked Immunosorbent Assay (ELISA), support the hypothesis that IL-33 is associated with Alzheimer's disease, either as a part of the disease mechanism or as an indicator of the body's attempt to combat the disease. These findings necessitate further research into the biological significance of IL-33 in Alzheimer's disease and its potential as a target for therapeutic intervention. The use of ELISA provides a robust method for such investigations, allowing for the accurate quantification of cytokine levels in clinical samples

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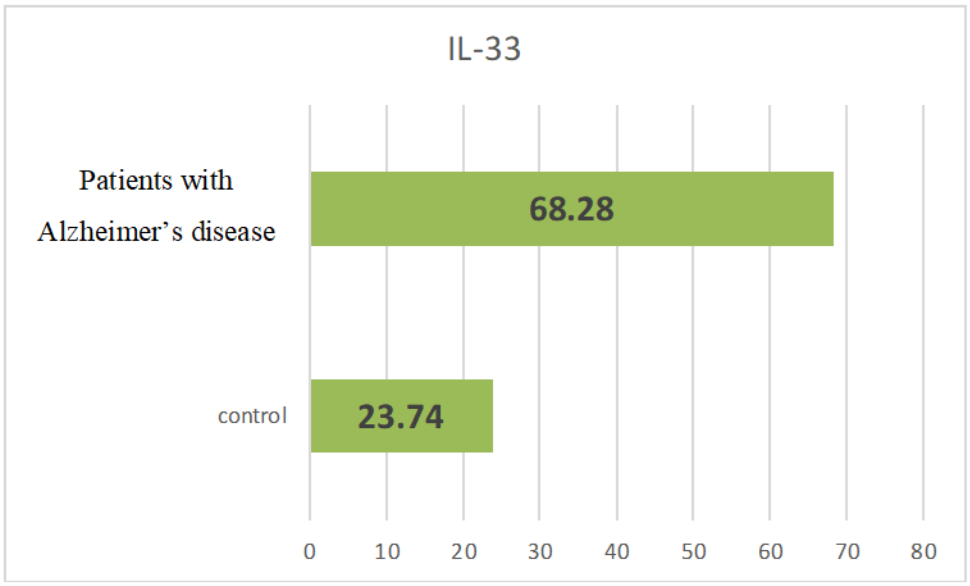

Figure 0-1; Comparative Analysis of Mean between Patients and Controls

4-DISCUSSION

The results presented in Figure 4.1 provide a clear indication that IL-33 levels are significantly elevated in patients with Alzheimer's disease compared to a healthy control group. With mean levels reaching 68.28 units in the patient group against 23.74 units in controls, there is a compelling suggestion that IL-33 is intricately linked with the pathophysiology of Alzheimer's disease (Jia *et al.*, 2023).

IL-33, a cytokine known to play roles in immune response modulation and inflammation, could be contributing to or reflecting the complex immunological environment associated with Alzheimer's disease. Its higher levels in patients suggest that IL-33 could be acting as an 'alarmin,' signaling tissue damage or stress and potentially influencing disease -immune system interactions. This is consistent with the dual role of IL-33 in both promoting and attenuating inflammatory responses, which can have varied implications in the disease microenvironment (Hu *et al*., 2011).

The use of ELISA for these measurements offers a high degree of specificity and sensitivity, reinforcing the reliability of these observations. However, while the data demonstrate an association between elevated IL-33 levels and Alzheimer's disease presence, the mechanism behind this elevation remains to be fully elucidated. It is unclear whether IL-33 contributes directly to the tumorigenic processes or if its levels rise as a consequence of the disease impact on surrounding tissues and the immune system (Piccoli *et al*., 2012).

Furthermore, the significant difference in IL-33 levels raises questions about the potential of IL-33 as a diagnostic biomarker for breast cancer. Its utility could extend to prognostic applications, possibly correlating with disease stage, aggressiveness, or response to treatment. Moreover, as an immune mediator, IL-33 might represent a novel therapeutic target, with strategies aimed at modulating its activity potentially impacting the course of the disease (Y. S. Kim *et al.*, 2017).

Future research should aim to clarify the role of IL-33 in Alzheimer's disease development and progression. Longitudinal studies could provide insight into how IL-33 levels change over the course of the disease and treatment. Additionally, mechanistic studies could explore how IL-33 interacts with other components of the tumor microenvironment and the immune system. The goal would be to determine whether IL-33's modulation can influence the clinical outcomes of breast cancer patients.

In conclusion, the elevated levels of IL-33 in Alzheimer's disease patients highlight a promising area of research that could have significant implications for the understanding and management of the disease. The potential of IL-33 as a biomarker and therapeutic target warrants further investigation to unlock new pathways for breast cancer diagnosis and treatment (Yang *et al.*, 2021).

CONCLUSION

In conclusion, the elevated levels of IL-33 in Alzheimer's disease patients highlight a promising area of research that could have significant implications for the understanding and management of the disease. The potential of IL-33 as a biomarker and therapeutic target warrants further investigation to unlock new pathways for Alzheimer's disease diagnosis and treatment.

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