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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 (1). 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 gets released when a cell is injured or undergoes stress and is an alarmin where it informs the immune system that something is damaged in the tissue or some other warning tone (2).
Regarding AD, it is believed that IL-33 regulates neuroinflammation which is being simultaneously a two edged sword in the advancement of the disease. On the one hand, IL-33 is capable of activating those signatures of genes which are related to tissue repair mechanisms and counter inflammation. Conversely, it may increase the inflammatory response thus causing additional nerve damage and worsening AD symptoms (3). It is important to establish the precise

contribution of IL-33 to AD inflammatory context extrapolated to the IL-33-mediated inflammatory context of AD.

give us useful information about the disease pathology and discover its possible treatment targets. Enzyme-Linked Immunosorbent Assay (ELISA) is the relatively specific and measurable method of detecting the level of IL-33 in biologic samples (e.g. cerebrospinal fluid (CSF) or blood serum) of AD patients. Through the measurement of IL-33, one can examine how it relates to the disease severity, levels of progression and responsiveness to treatment (4). The high sensitivity and specificity of ELISA method is a good option in this task since it is able to detect accurate concentrations of cytokines such as IL-33, even when they are low. This plays a major role in AD as increase or decrease of these levels by subtle changes in cytokines can lead to severe consequences as regards to the diseases progress as well as outcomes of a diseased patient (5).

Hence, the concept of IL-33 as a regulator of the neuroinflammation process in Alzheimer disease with the assistance of an ELISA technique is a potential field of study. It can expand our knowledge of inflammatory mechanisms in AD and may open new avenues of possible neuroinflammation-based therapeutic directions that, in theory, can stop or change the flow of the disease (6).

Under the purpose of isolation, IL-33 in Alzheimer disease was identified as an AD modulator using an ELISA method. It has the possibility of increasing our knowledge of the inflammatory mechanisms in AD and can lead to new therapeutic approaches to inflammation, which can slow or change the nature of the disease and the effects of IL-33 in brain of AD patient and non-AD patient.

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

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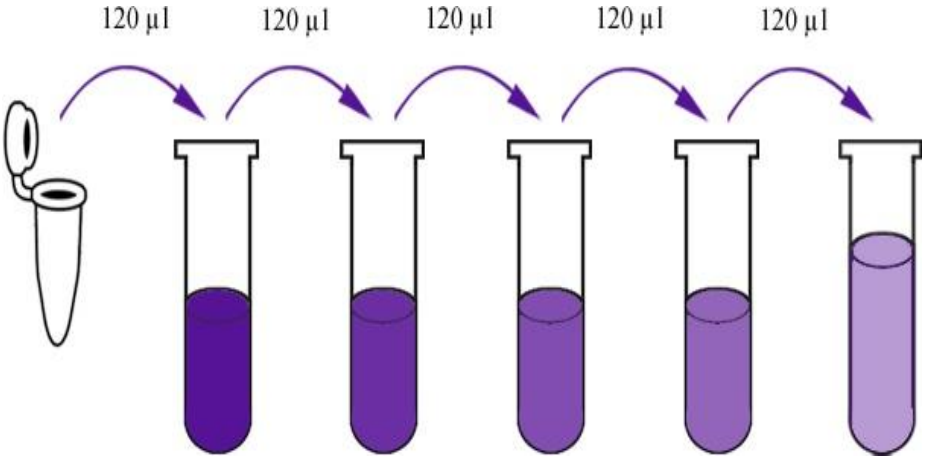

Figure 3-1 Dilutions preparation

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.

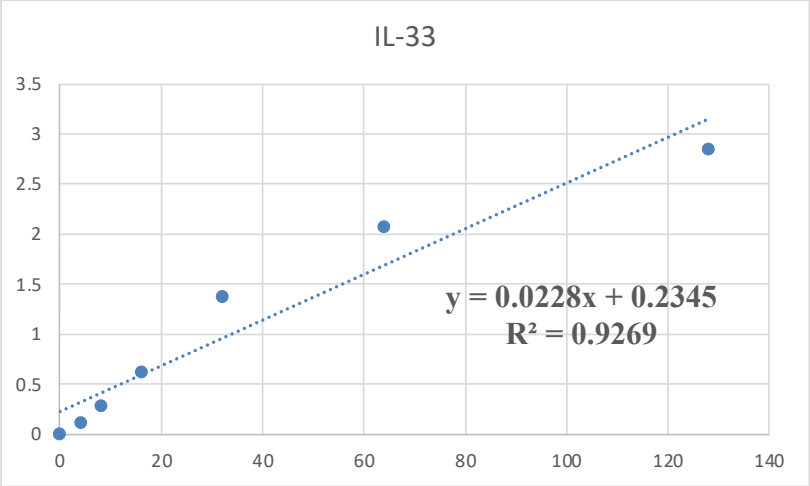

Figure 3-2. Standard Curve

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. High levels of IL-33 in patients may be manifested by the effect of IL-33 on regulating immune response in the microenvironment of the disease, which may play a role in inflammation during the development of Alzheimer disease or body response to disease develop.

These results obtained by the aid of Enzyme-Linked Immunosorbent Assay (ELISA) confirm the hypothesis that IL-33 can be linked to Alzheimer disease, whether it is part of the disease process, or as an indication of the body attempts at fighting the disease. These results require an additional study of the biological role of IL-33 in Alzheimer and its usage as a target of pharmacological intervention. Such studies can be substantiated by means of ELISA which enables one to determine the level of cytokines in clinical samples appropriately.

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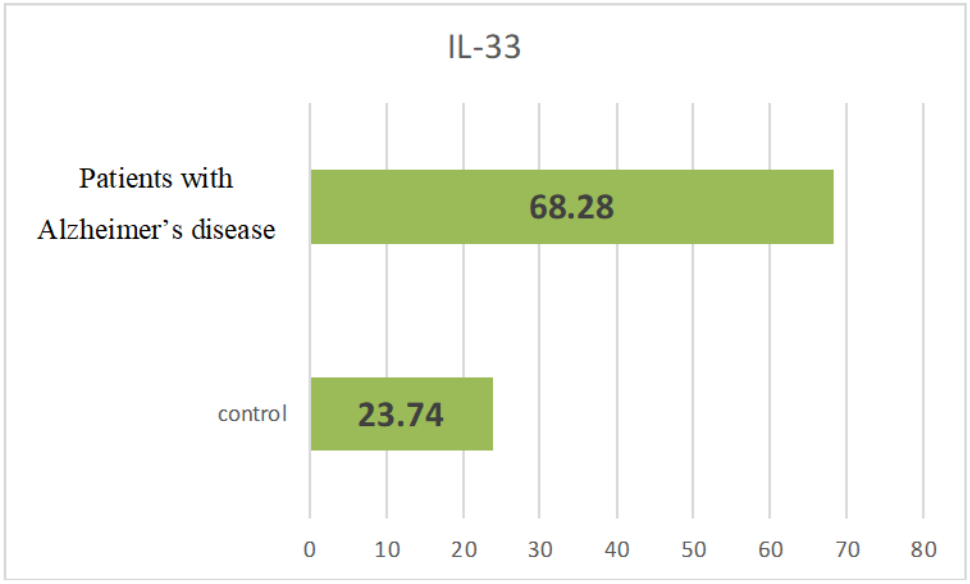

Figure 0-1; Comparative Analysis of Mean between Patients and Controls

4-DISCUSSION

The findings, as indicated in Figure 4.1 show a concrete clue that indeed the level of IL-33 is maximally inflated in patients with Alzheimer as against a healthy control group. Most individuals in the patient group had mean levels equalling 68.28 units versus 23.74 units in controls, which strongly recommends the notion that IL-33 is closely connected with the etiology of Alzheimer disease (7).

IL-33 is a cytokine that has been shown to be involved with immune response modulation and inflammation, which may be involved or indicative of a complex immunological environment surrounding AD. Its elevated concentrations within the patients mean that perhaps IL-33 is serving as an alarmin, as a messenger indicating tissue injury or distress, which may have an impact on the relationship between the disease and immune system. It is in agreement with the bivalent effect of IL-33 that mediates an increase and a decrease in inflammatory responses and can produce different effects in the disease microenvironment (8).

The reliability of these observations is backed by the fact that ELISA used to measure these quantities has a high specificity and sensitivity. Nevertheless, although the data allow concluding about the presence of an association between the increase in IL-33 levels and the presence of Alzheimer syndrome, the mechanism of its increase is still not finally clarified. There is no clear indication whether the IL-33 plays a direct role in the tumorigenic process or its levels are increased as a result of the disease effects on other tissues and the immune system (9).

Moreover, there is a very high possibility of IL-33 biomarker as a diagnostic biomarker in the risk of having cancer of the breast due to the great variation in the expression of IL-33. Its applicability may go all the way to prognostic situations, perhaps even correlating with level of disease, aggressiveness, or responsiveness to treatment. In addition, being an immune mediator, IL-33 may become a new target of therapy, and plans to regulate its activity may have a consequence on the disease process (10).

In further studies, the role of IL-33 in the development and progression of Alzheimer disease should be explained. Probably longitudinal studies will be able to show how IL-33 concentrations will change throughout the disease and treatment. Additionally, mechanist modeling may investigate the effect of IL-33 interaction with the tumor microenvironment and immune system components. The objective would be establishing the propensity towards the modulation of IL-33 effects in changing the clinical outcomes of patients with breast cancer.

In sum, it is important to note that IL-33 was found to be more abundant in patients with Alzheimer than in other patients, which points to one of the few promising directions that may play a critical role understanding and treating the disease. The future of IL-33 in diagnosis and treatment of breast cancer in terms of biomarker and therapeutic targets should be explored to open new possibilities in diagnosis and therapy of breast cancer (11).

CONCLUSION

As a conclusion, the high levels of IL-33 among patients of Alzheimer disease is a research whose significance seems promising though it can be so important in mapping out the disease. Possibility to

use IL -33 as biomarker and future treatment target should be explored further to open new horizons in Alzheimer diagnosis and treatment.

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