Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(2), 444-449 | ISSN:2251-6727

Understanding the Diagnostic Value of Inflammatory Cytokines TNF-α, IL-1β, and IL-6 in Alzheimer's Disease

Tara D Sharma [1], Ravi Kumar Sharma [2], Sudhir Sharma [3]

[1.] Department of Biotechnology University institute of biotechnology , Chandigarh university ,Mohali Punjab
[2.] Department of Biotechnology University institute of biotechnology, Chandigarh university, Mohali Punjab 140413
[3.] Department of Neurology Indira Gandhi Medical College & Hospital Shimla Himachal Shimla 171006

(Received: 16 January 2025 Revised: 20 February 2025 Accepted: 30 March 2025)

KEYWORDS

Alzheimer 's disease, Mini Mental State Examination, TNF-α, Interleukin-1β, Interleukins -6

ABSTRACT:

Alzheimer's disease(AD) is a progressive brain disorder that slowly deteriorates memory, thinking, and reasoning abilities eventually damage the capacity to do simple tasks. Dementia is mainly caused by it. Dementia is a set of brain degenerative illnesses that affect memory, thinking, and daily duties. These disorders are defined by nerve cell breakdown and brain injuries. Dementia primarily affects older persons and is not a normal component of aging. The aim of the current study was to investigate the role of pro-inflammatory cytokines in the diagnosis of AD. 30 patients diagnosed with AD were recruited and compared with 10 healthy control, utilizing the Mini Mental State Examination score (MMSE). AD was evaluated and staged. Patients' cognitive scores were considerably lower than the control mean. Tumor Necrosis Factor α, interleukins -1β and interleukins -6, were measured in human serum using sandwich Enzyme Linked Immuno sorbent Assay (ELISA) technique. Serum levels of all three markers were measured, and the results showed that levels of the three biomarkers were higher in AD patients when compared with control group.

1.1 Introduction

AD is a biological process that began with the growth of amyloid plaques and neurofibrillary tangles in the brain. AD is the leading cause of dementia that effects memory, thinking, and behavior [1]. AD is considered a worldwide health priority by the World Health Organization(WHO). The most recognized risk factor is old age, and the majority of Alzheimer's patients are 65 and older. AD is referred to as younger-onset Alzheimer's when it hit someone under the age of 65. Younger-onset dementia is known as early-onset AD [2]. Globally it is an estimated 57 million people have dementia and by 2050 that number is expected to rise to 153 million [3]. According to reports from India, there are 8.8 million old people living with dementia [4]. Dementia prevalence was higher among females than males, as well as in rural than urban areas [5]. There are three types of dementia i.e. vascular dementia, levy body dementia, frontotemporal [6].

In older adults, AD and Parkinson's disease are the utmost prevalent neurodegenerative illnesses. The basic mechanisms and causation of chronic neuronal cell death are still unknown, despite the fact that numerous theories have been put out [7]. It is now generally acknowledging that the etiology of both AD and PD disorders involves immune related events [8].

IL-1β isoforms are essentially homologous with a similar tertiary structure [9]. IL-1β release in the brain causes neuroinflammation by activating microglial cells, which produce neurotoxic chemicals and promote neuronal death furthermore. IL-1β promotes neurodegeneration by stimulating immune cells including monocytes and leukocytes [10]. AD proceeds microglia activate and concentrate around Aβ plaques, promoting phagocytosis. Microglia are the principal source of proinflammatory cytokine IL-1β in the AD [11].

IL-6 is one of the most consistent biomarkers discovered in both animal models and clinical research. A helical glycoprotein called interleukin-6 is important in neurodegeneration and neuroinflammation. It is intricately involved in controlling cognitive function. [12] Vascular IL-6 secretion levels were discovered to be

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(2), 444-449 | ISSN:2251-6727

considerably elevated in both mild and moderately severe AD. This could explain the presence of acute-phase proteins in their serum [13,14].

TNF-α is an essential proinflammatory cytokine which is increased in AD patients and interacts with AD genes. Several TNF-α promoter polymorphisms which enhance expression have been connected to inflammatory and infectious disorders. TNF-α increases in Amyloid plaques in postmortem brains of AD patients, as well as in plasma and cerebrospinal fluid which leads to disease progression and cognitive loss [15,16].

Biomarkers play important role in diagnostic accuracy in AD. Biomarkers convey diagnostically relevant information even during early disease stages. Recent technological advances allow very accurate and stable measurements of biomarkers utilizing fully automated procedures. Blood biomarkers having potential for application and screening tools for neurodegeneration and brain amyloid [17]. Our aim was to analyze the levels of TNF-α, IL -1β & IL-6 to find their association with AD.

1.1.1.Material and Methods

It was a case control and prospective study conducted at Indira Gandhi Medical College & Hospital Shimla H.P INDIA. All the subjects were recruited between (November 2021-March 2024). The protocol was authorized by the institute of ethical committee and review board at IGMC & hospital with reference number: ECR/533/INST/HP/2014/RR-20. We enrolled 30 patients with AD and 10 healthy control. We categorized the patients on the basis of following two criteria: radiological evidences and Mini Mental State Examination (MMSE) report of Alzheimer patients. We included patients who were more than 40 years of age and who gave written consent form was included. We excluded patients with active malignancy. 5 ml Venous Blood Sample was drawn aseptically by venipuncture and serum samples was prepared using standard procedures of preparation of samples for clinical laboratory analysis. All vials were centrifuged at 3000 rpm (round per minutes) for 10 minutes. Aliquoted samples were stored at -80°C as far as further analysis. We performed sandwich ELISA of IL-6, IL-1β & TNF-α on Thermofisher Scientific ELISA Reader. The micro titer well was coated with monoclonal antibody (M Ab 1) and M Ab 2 was labeled with horseradish peroxidase (HRP), unbound enzyme-labeled antibodies are eliminated after washing the micro titer plate. The chromogenic reaction is used to assess bound enzyme-labelled antibodies. Following the addition, chromogenic solution (TMB) is incubated. The reaction was stop by adding stop solution. Optical density was measured on valid wavelength 450nm. Human interleukins 1 β ELK Biotechnology ELISA kit was used for this experiment.

1.1.2Assessment of severity MMSE

MMSE was conducted for both the patient and control groups. The MMSE grades are server dementia, moderate impairment, (MCI) early onset (EOAD) distribution of patients according to MMSE were given in Table 1

1.1.3. Statistical analysis

An unpaired t test was used to compare TNF-α, IL-1β & IL-6 concentration between the patients and healthy controls, a comparison of mean TNF-α, IL-1β & IL-6 serum based on MMSE grade was also made. The significance threshold was established at p value <0.05. Blanks were used to measure the samples and standards. Each ELISA experiment had a minimum of seven standards p-value, OR, and 95%CI was calculated by using OpenEPI software. The result expressed as mean SD, median percentage.

1.1.4 Result

We recruited thirty patients having AD and then we compared these patients with ten healthy control in this current case control study, the mean age of the subjects 73.3±7.4, a male/female ratio of 15/15, and an MMSE score of 14.6±5.2 of the AD patients, 16% were urban and 83% were rural, Education years of the AD subjects were 4.2±4.03 and the control group was 2.3±1.9. demographic characteristics of the Group I and Group II is also listed in Table 5. The values are shown as a number (%) or as the mean, standard deviation. MMSE scores with interleukins levels in the blood. MMSE scores and blood levels of IL-1β (p =0.001, TNF-α (p = 0.008) or IL-6 (p = 0.261) in AD participants it showed result as statistically significant with IL-1β and TNF-α and statistically non-significant with IL-16 (Table 6).

Table 1: Distribution of patients according to MMSE scores

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(2), 444-449 | ISSN:2251-6727

MMSE Grade	Total number of patients (n=30)
Severe dementia (>9)	04 (12.9 %)
Moderate impairment (10-19)	22 (70.9%)
Mild cognitive impairment (20-25)	04 (12.9%)

The distribution of patients according to their MMSE grade is shown in the above table.12.9% of cases were classified as having severe dementia, whereas 70.9% of cases were diagnosed with mild dementia and 12.9% cases were found in early stage Alzheimer patients.

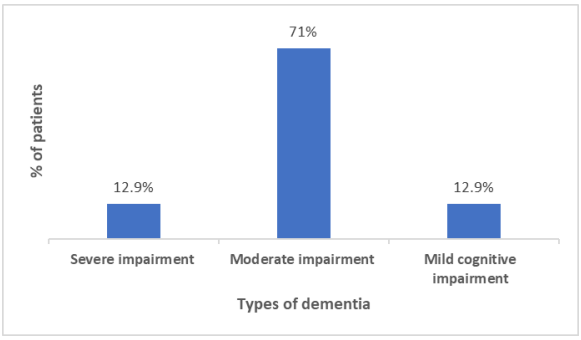

Figure 1.: Distribution of patients according to MMSE scores

IL-6 was analyzed using sandwich ELISA technique and kit was found that 30% patients were having elevated levels of IL-6 & 70% were having normal interleukins level. when both the groups were compare p value was 0.11 (non-significant), and odds ratio was 8.57, 95% confidence interval was 0.4507- 163. Thus we found that IL -6 has no association patients with dementia (table 2., figure 2)

Table 2. Comparison of IL-6 in case and control

IL-6	Case n=30(%)	Control n=10(%)	p value 0.11 OR 8.57 95% CI=0.4507-163
Elevated	9 (30%)	0 (0%)	
Normal	21(70%)	10 (100%)	

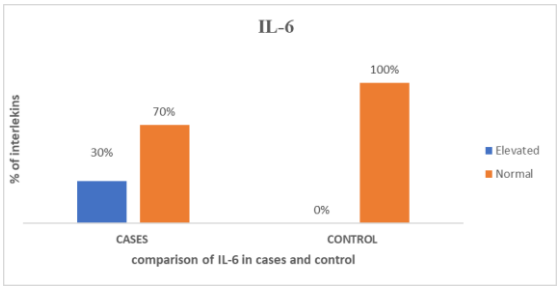

Figure2.

TNF-α was analyzed using sandwich ELISA technique and kit was found that 43% patients were having elevated levels of IL-6 & 56% were having normal interleukins level. when both the groups were compare p value was 0.02 (significant), and odds ratio was 15.29, 95% confidence interval was 0.8163-286.5. Thus TNF-α has association with dementia patients (table 3., figure 3)

Table 3. Comparison of TNF-α in case and control

TNF-α	Case n=30(%)	Control n=10(%)	p value 0.023 OR 15.29 95% CI= 0.8163 - 286.5
Elevated	13(43%)	0 (0%)	
Normal	17(56%)	10(100%)	

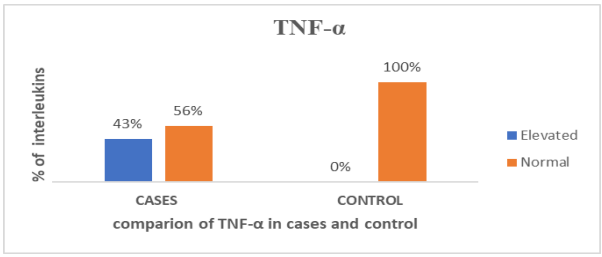

Figure 3

IL-1β was analyzed using sandwich ELISA technique and kit was found that 60% patients were having elevated levels of IL-1β & 40% were having normal interleukins level. when both the groups were compare p value was 0.002 (significant), and odds ratio was 30, 95% confidence interval was 1.59-563.2. Thus IL-1β has association with dementia patients (table 4, figure 4)

Table 4. Comparison of IL- 1β in case and control

IL -1β	Case n=30(%)	Control n=10(%)	p – value= 0.002 OR=30 95% CI= 1.59 - 563.2
Elevated	18(60%)	0(0%)	
Normal	12(40%)	10(100%)	

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(2), 444-449 | ISSN:2251-6727

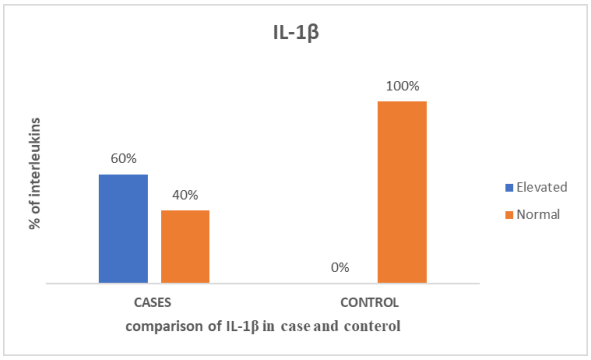

Figure:4.

Table:5. Demographic characteristics of Group1 (Cases)or Group II (controls) The age, rural urban, education presented as the Mean, SD or percentage. The parenthesis indicates the number of subjects in each group.

	Group I(AD patients n= 30)	Group II (Controls n = 10)	p value
Male	15(50%)	2(20%)	0.11
Female	15(50%)	8 (80%)	95%CI= 0.73-22.0
Education (years)	4.2±4.03	2.3±1.9	
Age mean ±SD	72±9.11	68.6±9.5	0.3181
Urban	16%	10%	
Rural	84%	90%	
Marital status	100%	99%	
Kids	93%	100%	
Household	84%	91%	
Employment	16%	9%	

Table:6 The comparison between MMSE and serum interleukins in AD patients.

Interleukins	p- value value		Result
IL- 6	19.54±23.3	0.2617	Not significant
IL -1β	107.9±94.6	0.001	significant
TNF-α	34.8±40.4	0.008	significant
MMSE	14.6±5.2		

1.1.5 Discussion

Our results shown that IL-1β considerably greater in AD patients' serum than in healthy controls by ELISA. These findings emphasize the role of IL-1β as a potential biomarker for neuroinflamation in AD. IL-β levels positively correlated with MMSE. These findings are compared with previous research (Forlenza OV 2010) they also found that IL-1β values were higher in people with AD [18]. Our results were similar to study by Dursun E et al. who found that IL-1β was significantly higher in AD patients [19]. IL-1β, IL-6, and TNF-α are examples of pro-inflammatory cytokines that affect the surrounding brain tissue. Recent research has shown that pro-inflammatory cytokine up-regulation has a variety of functions in both neurodegeneration and neuroprotection [20]. These results are consistent with Khemka VK et al. which also reported elevated IL-1β in AD patients compared with controls [21] but differ from Di Iorio A et al. which found no significant differences variations in sample size, patient demographics, or assay sensitivity could be the cause of these disparities [22]. The significant spike in IL-1β levels. suggests that it could be applied as an AD diagnostic biomarker.

our study shows that the serum levels of TNF-α was slightly higher in AD patients compare to healthy control, these results highlight how systemic inflammation plays a part in the pathophysiology of AD. TNF-α as a possible indicator of disease activity, TNF-α is linked to the detrimental effects of Aβ on synaptic memory processes and learning and memory disorders in AD. [23] Numerous studies have reported elevated inflammatory cytokines in AD patients. Chen R et al. (2012) reported elevated serum TNF-α [24]. TNF-α is overexpressed in AD patients' brains, especially in areas

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(2), 444-449 | ISSN:2251-6727

where amyloid-beta (Aβ) plaques are present. Kim YS et al. has found no association between Alzheimer & TNF-α [25].We found no significant difference in IL-6 levels between AD patients and healthy controls. Our findings are similar with other study by Richartz E et al. (2005), Wennström M et al. (2015) who found that Serum IL-6 levels was normal in AD patients [26,27]. However, contrary to the khemta et al. (2014), Dursun E et al. (2015), Yerman et al. (2021) found significant relationship between Alzheimer and IL-6 in human serum [28,29,30].

Conclusion:

In conclusion AD patients have slightly elevated levels of TNF α comparatively in healthy control, IL-1β was higher than IL -6 or TNF α in Alzheimer patients comparatively healthy control. It should be noted as well first the sample size was small, particularly when comparing the data between AD groups with control group which has prevented our ability to further subgroup the AD patients based on disease severity (e.g., MMSE scores) and then reanalyze the data, second, the standard deviations in serum interleukins was high in subjects Third limitation was medication history. Patients was already on medication before blood collection. So we should collect the blood sample before start the medication for accurate results.

Acknowledgement

The authors are grateful to thank department of Biotechnology, Chandigarh University, department of Neurology, IGMC Shimla for their required support.

Funding: None

Conflict of Interest: None

References

1. Babić Leko, M., Nikolac Perković, M., Klepac, N., Štrac, D. Š., Borovečki, F., Pivac, N., ... & Šimić, G. (2020). IL-1β, IL-6, IL-10, and TNF α single nucleotide polymorphisms in human influence the susceptibility to Alzheimer's disease pathology. *Journal of Alzheimer's Disease*, *75*(3), 1029-1047.
2. Sinyor, B., Mineo, J., & Ochner, C. (2020). Alzheimer's disease, inflammation, and the role of antioxidants. *Journal of Alzheimer's disease reports*, *4*(1), 175-183.
3. Chen, S., Cao, Z., Nandi, A., Counts, N., Jiao, L., Prettner, K., ... & Bloom, D. E. (2024). The global macroeconomic burden of Alzheimer's disease and other dementias: estimates and projections for 152 countries or territories. *The Lancet Global Health*, *12*(9), e1534-e1543.
4. Bhasin, S., & Kumar, B. (2024). Prevalence of Dementia in India. In *AI and Neuro-Degenerative Diseases: Insights and Solutions* (pp. 91-103). Cham: Springer Nature Switzerland.
5. Choudhary, A., Ranjan, J. K., & Asthana, H. S. (2021). Prevalence of dementia in India: A systematic review and meta-analysis. *Indian Journal of Public Health*, *65*(2), 152-158.
6. Mirzaei, G., & Adeli, H. (2022). Machine learning techniques for diagnosis of alzheimer disease, mild cognitive disorder, and other types of dementia. *Biomedical Signal Processing and Control*, *72*, 103293.
7. Yu, Z., Luo, H., Fu, W., & Mattson, M. P. (1999). The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. *Experimental neurology*, *155*(2), 302-314.
8. Chan, A. H., & Schroder, K. (2019). Inflammasome signaling and regulation of interleukin-1 family cytokines. *Journal of Experimental Medicine*, *217*(1), e20190314.
9. Lopez-Rodriguez, A. B., Hennessy, E., Murray, C. L., Nazmi, A., Delaney, H. J., Healy, D., ... & Cunningham, C. (2021). Acute systemic inflammation exacerbates neuroinflammation in Alzheimer's disease: IL-1β drives amplified responses in primed astrocytes and neuronal network dysfunction. *Alzheimer's & Dementia*, *17*(10), 1735-1755.
10. Facci, L., Barbierato, M., Zusso, M., Skaper, S. D., & Giusti, P. (2018). Serum amyloid A primes microglia for ATP-dependent interleukin-1β release. *Journal of Neuroinflammation*, *15*, 1-11.
11. Facci, L., Barbierato, M., Zusso, M., Skaper, S. D., & Giusti, P. (2018). Serum amyloid A primes microglia for ATP-dependent interleukin-1β release. *Journal of Neuroinflammation*, *15*, 1-11.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(2), 444-449 | ISSN:2251-6727

12. Hager, K., Machein, U., Krieger, S., Platt, D., Seefried, G., & Bauer, J. (1994). Interleukin-6 and selected plasma proteins in healthy persons of different ages. *Neurobiology of aging*, *15*(6), 771-772.
13. Cojocaru, I. M., Cojocaru, M., Miu, G., & Sapira, V. (2011). Study of interleukin-6 production in Alzheimer's disease. *Rom J Intern Med*, *49*(1), 55-8.
14. Perry, R. T., Collins, J. S., Wiener, H., Acton, R., & Go, R. C. (2001). The role of TNF and its receptors in Alzheimer's disease. *Neurobiology of aging*, *22*(6), 873-883.
15. Serafini, S., Ferretti, G., Monterosso, P., Angiolillo, A., Di Costanzo, A., & Matrone, C. (2024). TNF-α Levels Are Increased in Patients with Subjective Cognitive Impairment and Are Negatively Correlated with β Amyloid-42. *Antioxidants*, *13*(2), 216.
16. Gunes, S., Aizawa, Y., Sugashi, T., Sugimoto, M., & Rodrigues, P. P. (2022). Biomarkers for Alzheimer's disease in the current state: a narrative review. *International journal of molecular sciences*, *23*(9), 4962.
17. Gunes, S., Aizawa, Y., Sugashi, T., Sugimoto, M., & Rodrigues, P. P. (2022). Biomarkers for Alzheimer's disease in the current state: a narrative review. *International journal of molecular sciences*, *23*(9), 4962.
18. Dursun, E., Gezen-Ak, D., Hanağası, H., Bilgiç, B., Lohmann, E., Ertan, S., ... & Yılmazer, S. (2015). The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. *Journal of neuroimmunology*, *283*, 50-57.
19. Wang, W. Y., Tan, M. S., Yu, J. T., & Tan, L. (2015). Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Annals of translational medicine*, *3*(10), 136.
20. Khemka, V. K., Ganguly, A., Bagchi, D., Ghosh, A., Bir, A., Biswas, A., ... & Chakrabarti, S. (2014). Raised serum proinflammatory cytokines in Alzheimer's disease with depression. *Aging and disease*, *5*(3), 170.
21. Di Iorio, A., Ferrucci, L., Sparvieri, E., Cherubini, A., Volpato, S., Corsi, A., ... & Paganelli, R. (2003). Serum IL-1β levels in health and disease: a population-based study.'The InCHIANTI study'. *Cytokine*, *22*(6), 198-205.
22. Tobinick, E. L., & Gross, H. (2008). Rapid improvement in verbal fluency and aphasia following perispinal etanercept in Alzheimer's disease. *BMC neurology*, *8*, 1-9.
23. Chen, R., Yin, Y., Zhao, Z., Huang, L., Huang, S., Zhuang, J., ... & Li, P. (2012). Elevation of serum TNF-α levels in mild and moderate Alzheimer patients with daytime sleepiness. *Journal of Neuroimmunology*, *244*(1-2), 97-102.
24. Kim, Y. S., Lee, K. J., & Kim, H. (2017). Serum tumour necrosis factor-α and interleukin-6 levels in Alzheimer's disease and mild cognitive impairment. *Psychogeriatrics*, *17*(4), 224-230.
25. Richartz, E., Stransky, E., Batra, A., Simon, P., Lewczuk, P., Buchkremer, G., ... & Schott, K. (2005). Decline of immune responsiveness: a pathogenetic factor in Alzheimer's disease?. *Journal of psychiatric research*, *39*(5), 535-543.
26. Wennström, M., Hall, S., Nägga, K., Londos, E., Minthon, L., & Hansson, O. (2015). Cerebrospinal fluid levels of IL-6 are decreased and correlate with cognitive status in DLB patients. *Alzheimer's research & therapy*, *7*, 1-8.
27. Khemka, V. K., Ganguly, A., Bagchi, D., Ghosh, A., Bir, A., Biswas, A., ... & Chakrabarti, S. (2014). Raised serum proinflammatory cytokines in Alzheimer's disease with depression. *Aging and disease*, *5*(3), 170.
28. Dursun, E., Gezen-Ak, D., Hanağası, H., Bilgiç, B., Lohmann, E., Ertan, S., ... & Yılmazer, S. (2015). The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. *Journal of neuroimmunology*, *283*, 50-57.
29. Yeram, N., Dalvi, S., Mankeshwar, R., Patil, V., Kale, V., Jagiasi, K., & Abichandani, L. (2021). Relationship between cortisol, Interleukin-6 and homocysteine in Alzheimer's disease. *Qatar Medical Journal*, *2021*(2), 33.