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Association of Plasma Amyloid-Beta and Phosphorylated Tau Biomarkers with Alzheimer's Disease

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Abstract :

Background:
Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to cognitive decline, dementia, and ultimately death. The neuropathological features such as amyloid-β (Aβ) plaques and phosphorylated tau (p-Tau) tangles. Blood based biomarkers of Aβ and p-Tau have emerged as promising tools for early diagnosis, monitoring, and risk stratification of AD.

Objective: This study aims to assess the role of blood-based amyloid-beta and phosphorylated tau biomarkers in monitoring Alzheimer's disease progression and enhancing risk stratification among individuals

Martials& method: A study ′s case – control was conducted at Marjan Medical City from April 2025-March 2026.A total of 100 blood samples were obtained from individuals with age ≥ ٦٠ years (male 40,Female 60) .They are seeing a neurologist, including 25 classified as healthy controls(non-carriers APOE ε4) (10M,15 F) and 75 diagnosed with Alzheimer's disease(APOE ε4 carriers) through PCR analysis.. Aβ1-42, Aβ1-40, p-Tau181, and p-Tau217 were measured by ELISA using commercial kits: Elabscience E-EL-H0543 for Aβ1-42, Elabscience E-EL-H0542 for Aβ1-40, Abcam ab325078 for p-Tau181, and Abcam ab318936 for p-Tau217. The Aβ42/Aβ40 ratio was calculated by

dividing Aβ1-42 by Aβ1-40. absorbance was measured with a Fisher Scientific ELISA plate reader.

Results: Conventional PCR analysis of 100 blood samples showed that 75 samples (75%) were positive for the APOE ε4 allele and were classified as APOE ε4 carriers, whereas 25 samples (25%) were negative and classified as non-carriers. ELISA analysis demonstrated significantly lower Aβ1-42 levels in Alzheimer's disease patients (4.02 ± 8.031 pg/mL) compared with controls (9.07 ± 10.43 pg/mL) ($p < 0.05$). Similarly, Aβ1-40 levels were significantly reduced in Alzheimer's disease patients (3.03 ± 6.01 pg/mL) compared with controls (7.08 ± 9.05 pg/mL) ($p < 0.05$).An Aβ42/Aβ40 ratio below 0.077 was considered amyloid-positive. And higher levels of p-Tau181 (4404.1 ± 5053 pg/mL) and p-Tau217 (44.02 ± 63.1 pg/mL) compare control(198.04±221,09),(20.12± 28.33)respectively, were observed. The results showed statistically significant differences between Alzheimer's disease patients and controls ($p < 0.05$).

Conclusion:

The biomarker levels were significantly associated with Alzheimer's disease. These findings suggest that combining APOE ε4 detection by PCR with ELISA-based blood biomarkers may support Alzheimer's disease risk assessment and differentiation from healthy controls.

Key words: Alzheimer's , Aβ, p-Tau.

Introduction:

Alzheimer's disease (AD) is the most common neurodegenerative disorder, characterized pathologically by extracellular deposition of β-amyloid (Aβ) into senile plaques and intracellular accumulation of hyperphosphorylated tau (pTau) as neurofibrillary tangles[1]. Clinically, AD patients show memory deterioration with varying cognitive dysfunctions. The major clinical features of AD are memory loss, cognitive dysfunction, and personality changes .Selective memory impairment is often the earliest clinical manifestation of AD but there is no cure for this disease, only treatments that are available aim to relieve the symptoms .AD occurs in two forms, early onset familial and late-onset sporadic; genetic mutations in PS1, PS2, and APP genes cause early onset familial AD, and a combination of lifestyle, environment and genetic factors causes the late-onset sporadic form of the disease. However, accelerated disease progression is noticed in patients with familial AD[2]. The Potential risk factors for Alzheimer's disease[3]. There are two types of risk factors for Alzheimer's disease that are modifiable and non-

modifiable factors. Modifiable risk factors mainly include diseases, brain injuries, unhealthy lifestyles, and environmental factors, and non-modifiable factors include age, gender, family history, and genetics. the digestion of APP is done by cumulative action of alpha- and gamma-secretases that produce insoluble peptides, amyloid-beta, which cluster together to form amyloid beta plaques thus degenerating cells[4,5] .In healthy brains, cleavage of Aβ is done by beta-secretase enzymes resulting in the formation of soluble APP fragments, and the rest of the APP is further cleaved by γ-secretase-producing peptides that are released outside the cell and rapidly removed/degraded. However, in elderly people, the secretase homeostasis is dysregulated, and APP is cleaved by β and γ-secretase and produces insoluble amyloid beta peptides. Amyloid beta (Aβ) is the cleavage product of a glycoprotein, amyloid precursor protein (APP), and is normally present in the brain as part of the signal transduction process [6,7]. The dysregulation of Aβ levels in the brain leads to the formation of senile plaque, and deposition of Aβ, which causes cognitional disabilities in patients with AD . APP is a transmembrane protein that has three domains: one inside the cell, one in the cell membrane, and one protruding out of the cell. The domains of APP are digested by alpha-, beta- and gamma-secretases[8]. APP cleavage by β-secretase followed by γ-secretase cleavage yields various lengths of Aβ peptides like $A\beta_{42}$ and $A\beta_{40}$ [9]. These two major isoforms of Aβ differ in the number of residues as $A\beta_{42}$ has two extra residues at its C-terminus as compared to $A\beta_{40.}$ The Aβ isoform that forms amyloid plaques in AD is mostly Aβ42, and some plaques only contain the Aβ42 isoform[10].

Materials & methods:-

A case–control study was conducted at Marjan Medical City from April 2025 to March 2026**.** The study included 100 patients aged 60 years and above, comprising 40 males and 60 females, who were diagnosed with Alzheimer's disease based on clinical examination. Fresh blood samples were collected from the patients for DNA extraction and molecular analysis**.** The control group consisted of 25 individuals who tested negative for Alzheimer's disease, and blood samples were also collected from them for DNA extraction and comparison with the control group.

Ethical approval was granted by the Committee of Publication Ethics at the Faculty of Medicine, Babylon University, Iraq (Ref. No. ١٢٨٠8, 28/0٤/202٥). Verbal consent was obtained from all participants.

Inclusion Criteria

Patients aged 60 years or older. Patients clinically diagnosed with Alzheimer's disease by a specialist physician. Patients with cognitive impairment compatible with Alzheimer's disease.

Exclusion Criteria

Patients with other types of dementia, such as vascular dementia, Parkinson's disease dementia, or frontotemporal dementia. Patients with a history of stroke, brain tumor, or severe head injury. Patients with acute or chronic inflammatory, autoimmune, or infectious diseases. Patients with severe liver, kidney, or heart disease. Patients receiving corticosteroids, immunosuppressive drugs, or chemotherapy. Patients with malignancy.

DNA extraction:-

Blood samples were stored in normal saline at -20°C prior to processing. DNA extraction was performed using the Geneaid Biotech DNA extraction kit (Presto, UK), following the protocol provided by the manufacturer. The extracted DNA was kept at -20°C until further analysis. DNA quality and concentration were assessed using a NanoDrop spectrophotometer (DeNovix, USA).

Detection of APOE genotyping through PCR analysis:

The PCR reactions were carried out with primers specific. DNA amplification was performed following the protocol of the One -Step PCR System (Promega). The final PCR reaction volume was 20 µl. APOE genotyping was performed by PCR amplification of APOE exon 4 using forward primer 5′-CGGCTGTCCAAGGAGCTG-3′ and reverse primer 5′-CCTGTTCCACCAGGGGC-3′, producing a 289 bp amplicon.

Measurement of Alzheimer's Disease Blood Biomarkers by ELISA

Blood samples were collected from all participants and serum/plasma was separated and stored at −20°C until analysis. The concentrations of Aβ1-42, Aβ1-40, p-Tau181, and p-Tau217 were measured using commercially available ELISA kits according to the manufacturers' instructions. Aβ1-42 was measured using Human Aβ1-42 ELISA Kit, Elabscience, catalog No. E-EL-H0543. Aβ1-40 was measured using Human Aβ1-40 ELISA Kit, Elabscience, catalog No. E-EL-H0542. Phosphorylated Tau181 was measured

using Human Tau phospho T181 ELISA Kit, Abcam, catalog No. ab325078. Phosphorylated Tau217 was measured using Human Tau phospho T217 ELISA Kit, Abcam, catalog No. ab318936. The Aβ42/Aβ40 ratio was calculated by dividing the concentration of Aβ1-42 by the concentration of Aβ1-40 for each participant.

Statistical analysis:-

Data were analyzed using SPSS software version 26.0. Continuous variables were expressed as mean ± standard deviation (SD)**,** while categorical variables were presented as frequencies and percentages**.** The normality of data distribution was assessed using the Shapiro–Wilk test**.** Differences in biomarker levels between Alzheimer's disease patients and controls were analyzed using the independent samples t-test for normally distributed data or the Mann–Whitney U test for non-normally distributed data. The association between APOE ε4 carrier status and Alzheimer's disease was evaluated using the Chi-square test**.** A p-value < 0.05 was considered statistically significant.

Results:-

Distribution of Alzheimer's patients and controls according to sex

Among the 100 participants, 75 were Alzheimer's disease patients and 25 were controls. The study included 40 males and 60 females. Among Alzheimer's disease patients, 30 were males and 45 were females, while the control group included 10 males and 15 females. No statistically significant association was observed between sex and Alzheimer's disease ($\chi^2 = 0.000$, $p = 1.000$)as in Table 1.

Table 1: Distribution of Alzheimer's patients and controls according to sex

Sex	Alzheimer's patients	Controls	Total		
Male	30	10	40	**$\chi^2 = 0.000$**	**p = 1.000**
Female	45	15	60		
Total	75	25	100		
No statistically significant association was found between sex and Alzheimer's disease (p > 0.05).					

Molecular detection of Alzheimer's disease by PCR

Out of 100 participants, approximately 75 patients were identified as carriers of the APOE gene. PCR amplification of the APOE gene yielded a specific amplicon of 289 bp**.**as in Figure **1.**

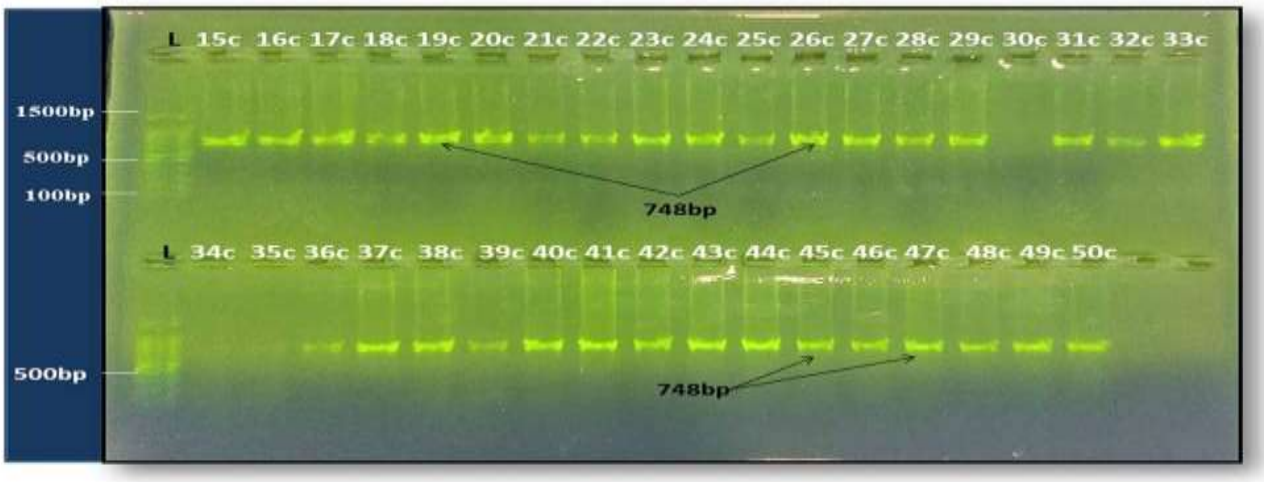

Figure 1. Gel electrophoresis analysis of PCR-amplified APOE gene products 748bp. A DNA ladder ranging from 500-1500 bp was used as a molecular size marker. Isolates 75 showed positive amplification results, indicating the presence of the APOE gene**.**

Comparison of Alzheimer's disease biomarkers between patients and controls

The results showed significant differences in Alzheimer's disease biomarkers between patients and controls. The mean levels of Aβ1-42 and Aβ1-40 were lower in Alzheimer's patients than in controls, while p-Tau181 and p-Tau217 levels were markedly higher in patients as in Table2. Both t-test and Mann–Whitney test confirmed that these differences were statistically significant, indicating that these biomarkers may be associated with Alzheimer's disease progression.

Table 2: Serum levels of Aβ1-42, Aβ1-40, p-Tau181, and p-Tau217 in Alzheimer's patients and controls

Marker	Patient Mean ± SD	Control Mean ± SD	t-test	Mann–Whitney	Results
Aβ1-42	4.02 ± 8.03	9.07 ± 10.43	0.0338	0.0002	Significant
Aβ1-40	3.03 ± 6.01	7.08 ± 9.05	0.0449	0.0138	Significant
p-Tau181	4404.10 ± 5053.00	198.04 ± 221.09	<0.0001	<0.0001	Significant
p-Tau217	44.02 ± 63.10	20.12 ± 28.33	0.0112	0.0010	Significant

Table3: **Evaluation of Biomarker Cutoff Values for Differentiating Alzheimer's Disease Patients from Controls**

Marker	AUC	Cutoff	Sensitivity	Specificity
Aβ1-42	0.751	≤ 1.057	57.3%	88.0%
Aβ1-40	0.665	≤ 1.367	68.0%	72.0%
p-Tau181	0.984	≥ 458.997	98.7%	92.0%
p-Tau217	0.720	≥ 6.247	100.0%	56.0%

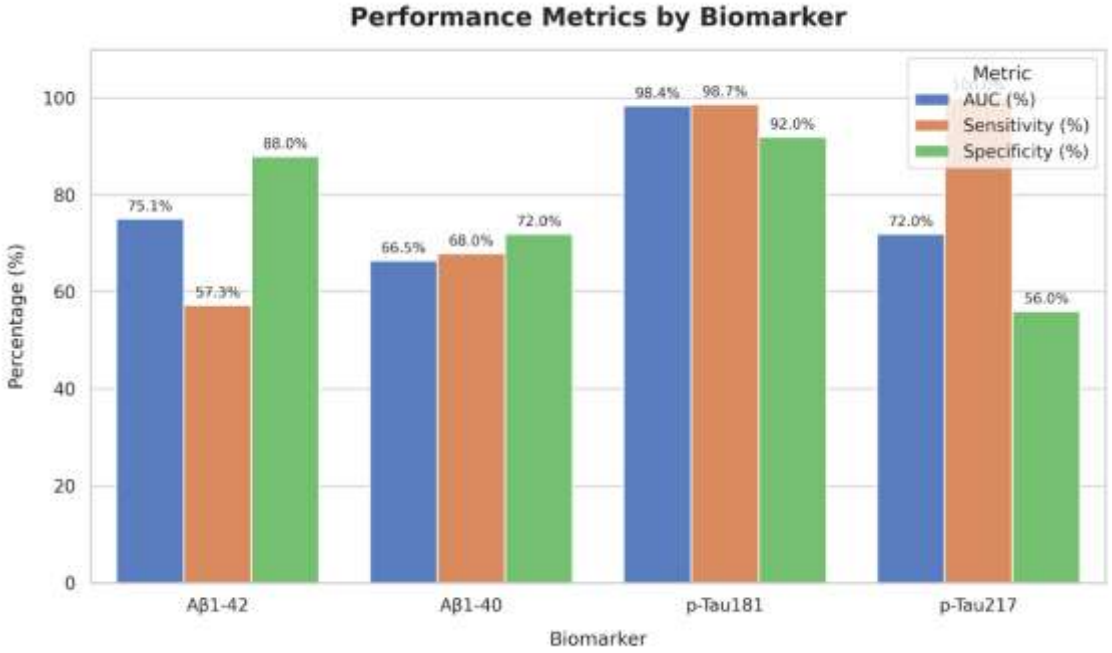

Figure2: Comparative diagnostic performance of Aβ1-42, Aβ1-40, p-Tau181, and p-Tau217 biomarkers based on AUC, sensitivity, and specificity.

Table4: Diagnostic Accuracy of Alzheimer's Disease Biomarkers Based on AUC, Sensitivity, and Specificity

Marker	**AUC**	**AUC 95% CI Low**	**AUC 95% CI High**	**Sensitivity**	**Specificity**
Aβ1-42	0.750933333	0.646928573	0.841612632	0.573333333	0.88
Aβ1-40	0.664533333	0.513499666	0.805989917	0.68	0.72
p-Tau181	0.984	0.95339847	1	0.986666667	0.92
p-Tau217	0.72	0.575169837	0.854300949	1	0.56

Discussion:-

The present study evaluated the association of sex, APOE gene detection, and circulating Alzheimer's disease (AD) biomarkers among 100 participants, including 75 AD patients and 25 healthy controls. The findings demonstrated significant alterations in serum biomarker levels among AD patients, highlighting their potential utility in disease diagnosis and progression monitoring.Regarding sex distribution, no significant association was observed between sex and AD occurrence ($p = 1.000$). Although females constituted a larger proportion of AD cases than males, this difference reflected the overall study population distribution rather than a true sex-related risk. This finding is consistent with recent studies reporting that sex alone may not significantly influence plasma AD biomarker levels after adjustment for demographic and clinical variables. A recent study by Zhang et al. (2025) found no significant correlation between sex and plasma p-Tau181 or p-Tau217 levels among AD patients and controls. However, other studies have suggested that females may exhibit greater susceptibility to AD pathology due to hormonal, genetic, and neuroinflammatory mechanisms, particularly in relation to tau pathology and APOE-related risk factors.

Molecular analysis demonstrated successful amplification of the APOE gene, producing a specific PCR product. APOE remains the strongest genetic risk factor for late-onset Alzheimer's disease, particularly the APOE ε4 allele, which has been linked to increased amyloid deposition, tau accumulation, and accelerated cognitive decline. Recent evidence indicates that APOE ε4 carriers show faster progression of plasma p-Tau217 levels and

more rapid disease advancement compared with non-carriers.The biomarker analysis revealed significantly lower levels of Aβ1-42 and Aβ1-40 in AD patients compared with controls. These findings support the amyloid cascade hypothesis, whereby circulating amyloid-beta concentrations decrease as peptides accumulate within cerebral amyloid plaques. Similar reductions in amyloid biomarkers have been reported in numerous AD studies and are considered characteristic pathological changes associated with disease progression. Lower plasma Aβ42 concentrations have been consistently associated with increased amyloid burden and cognitive impairment.Conversely, p-Tau181 and p-Tau217 levels were significantly elevated in AD patients. Phosphorylated tau proteins are considered highly specific markers of neuronal injury and neurofibrillary tangle formation. The remarkably higher levels observed among patients in the current study are consistent with recent reports identifying p-Tau217 and p-Tau181 as among the most accurate blood-based biomarkers for AD diagnosis. Recent investigations have demonstrated that p-Tau217 rises early in the disease process and closely reflects both amyloid and tau pathology. Furthermore, elevated p-Tau181 and p-Tau217 levels have been strongly associated with cognitive decline and disease severity.Receiver operating characteristic (ROC) analysis demonstrated superior diagnostic performance for p-Tau181, which achieved the highest AUC (0.984), sensitivity (98.7%), and specificity (92%). These findings are in agreement with recent studies reporting excellent diagnostic accuracy of plasma p-Tau181 for distinguishing AD patients from cognitively normal individuals. Although p-Tau217 achieved 100% sensitivity in the current study, its specificity was lower (56%), suggesting that while it may be highly useful as a screening biomarker, p-Tau181 may provide greater overall diagnostic precision. Recent literature similarly highlights p-Tau217 and p-Tau181 as leading blood-based biomarkers, with p-Tau217 often showing superior sensitivity for early pathology detection and p-Tau181 providing robust diagnostic discrimination.

Overall, the present findings support the growing evidence that blood-based biomarkers, particularly p-Tau181 and p-Tau217, represent valuable tools for the diagnosis and monitoring of Alzheimer's disease. The combination of APOE genetic assessment with plasma biomarker analysis may further improve risk stratification, early detection, and clinical management of AD patients.

Conclusion:-

This study showed significant differences in serum levels of Aβ1-42, Aβ1-40, p-Tau181, and p-Tau217 between Alzheimer's disease patients and controls. Alzheimer's patients had lower Aβ1-42 and Aβ1-40 levels and higher p-Tau181 and p-Tau217 levels. Among these biomarkers, p-Tau181 demonstrated the highest diagnostic accuracy, sensitivity, and specificity. These findings suggest that amyloid-beta and phosphorylated tau biomarkers

may be associated with Alzheimer's disease progression and could be useful for diagnosis and monitoring.

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Authors' contributions

The authors conceived and designed the experiment performed the experiment, analyzed the data participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

No conflicts of interest**.**

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