Evaluation of Salivary Proteomic and Genomic Biomarkers as Non-Invasive Diagnostic Tools for Early Detection of Alzheimer's and Parkinson's Diseases: A Schematic Assessment and Meta-Analysis

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ABSTRACT

Background: Salivary biomarkers are non-invasive molecules that indicate neurodegenerative illnesses, especially Alzheimer disease (AD) and Parkinson's disease (PD). This study was conducted to determine the diagnostic precision of salivary proteomic and genomic biomarkers in terms of early AD and PD detection.

Methods: A systematic literature search was conducted in PubMed, Web of Science, and Google Scholar, and studies were included from 2016 to 2025. Research that examined salivary biomarkers in AD and PD was eligible. The data were analyzed with a random-effects model, and odds ratios (OR), standard mean differences (SMD), and 95% confidence intervals (CI) were estimated. Also, subgroup and sensitivity analyses were performed. To assess the risk of bias, the Newcastle-Ottawa Scale (NOS) was applied for the included observational studies.

Results: A total of 11 eligible studies concerning proteomic biomarkers, including amyloid-β (Aβ42, A β40) and alpha-synuclein total (a-synTotal) and alpha-synuclein Oligomer (a-synOligo), and genomic biomarkers like different salivary microRNAs, were included. Meta-analysis indicated that A β42 (OR=0.70; 95% CI: 0.41 to 1.1) and Aβ40 (OR=1.01; 95% CI: 0.97 to 1.06) had significant discriminatory potential in AD patients; but a-synOligo (SMD = 2.90; 95% CI: -0.59–6.39) and a-synTotal (SMD = 0.44; 95% CI: -3.14 to 4.02) was higher in PD patients as compared with controls. Genomic biomarkers demonstrated inconsistent findings (SMD = -0.18; 95% CI: -1.79–1.42) because of difference in microRNA types. Heterogeneity was high (I2 > 90%), which is caused by alterations in study design and in the methods to measure biomarkers.

Discussion: Salivary biomarkers were found to be an insignificant yet exceptional method of early examination of AD and PD. Nonetheless, the inconsistency of different studies points to develop standardized protocols.

Keywords: Alzheimer's Disease / Diagnosis, Parkinson's Disease / Diagnosis, Biomarkers, Biological / Analysis, Saliva / Chemistry, Proteomics.

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INTRODUCTION

Neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD) are significant global public health issues, particularly as populations are aging all over the world [1]. In AD, cognitive deterioration, memory loss, and functional impairment gradually worsen [2]. However, PD is mostly associated with motor symptoms, namely tremors, rigidity, and bradykinesia, as well as non-motor symptoms, such as cognitive impairment [3]. The incidence of both disorders continues to rise, and their impact on the healthcare system, care providers, and quality of life is high [4].

The effective management of AD and PD is highly dependent on early and accurate diagnosis, especially to implement the measures that can slow down the progression of the disease or improve the management of the symptoms [5]. Nevertheless, the existing diagnostic practices are based on the use of neuroimaging, cerebrospinal fluid (CSF), and clinical diagnostics, all of which are invasive, expensive, or lacking sensitivity in detecting the disease in its early stages [6]. Such restrictions have led to the need to find alternative non-invasive diagnostic methods that are credible, convenient, and capable of identifying pathophysiological changes related to the disease at an early level [7].

Saliva is a non-invasive and potential bio fluid in diagnostic research [8]. It possesses a complex composition of proteins, microRNA (miRNAs) and extracellular vesicles which represent the systemic and neurological events [9]. A number of salivary biomarkers, including a-synuclein, Aβ42, phosphorylated tau (p-tau181), miR-153 and miR-223 have demonstrated the potential of distinguishing between AD or PD patients with healthy individuals [10]. Although findings are encouraging, biomarker selection, study design including the diagnostic accuracy of biomarkers varies widely across studies, and it is not possible to reach unified conclusions [11].

The primary goal of the proposed article is a systematic review and meta-analysis of the existing evidence of salivary proteomic and genomic biomarkers as non-invasive biomarkers to diagnose Alzheimer and Parkinson diseases at an early stage. The purpose of this study is to evaluate their diagnostic capabilities, undergo analysis of the differences in biomarker differences, and offer a schematic construct of the future of translational research into non-invasive methods of neurodegenerative disease diagnostics.

METHODS

Study Design

This systematic review and meta-analysis was carried out based on PRISMA 2020 guidelines [12]. The study aimed at synthesizing observational and cross-sectional studies that assessed the diagnostic value of salivary proteomic and genomic biomarkers in early diagnosis of Alzheimer Disease (AD) and Parkinson Disease (PD). The main focus was on the assessment of quantitative measures like difference in mean values of the biomarker expression levels, in place of sensitivity or specificity.

Inclusion Criteria

The inclusion criteria were that the studies had to be original peer-reviewed articles published in English during 2016-2025, measured salivary biomarkers (proteomic or genomic) and included human participants clinically diagnosed with AD or PD. The included studies should have provided at least quantitative data of biomarkers (means, standard deviations, or other statistical results) that can be used in meta-analysis.

Exclusion Criteria

Articles that were reviews, meta-analyses, conference abstracts, editorials, case reports and animal-based studies were excluded. Moreover, the studies examining the biomarkers of other biological fluids, e.g. CSF, blood, or urine were not included. Those articles that did not provide enough quantitative data or contained non-accessible full text were removed as well.

Literature Search

An extensive search was conducted in PubMed and Google Scholar from 2016 to 2023. The search was done using Boolean operators and key words: "Alzheimer S Disease" OR "Parkinson S Disease"; saliva OR salivary biomarkers; proteomics OR genomics OR microRNA OR a-synuclein OR amyloid-β OR tau. Manual search of reference lists of the included studies was also performed to determine additional relevant papers.

Study Selection

The process of selection took two steps. At first, titles and abstracts were used to eliminate irrelevant studies. Afterwards, the full-text articles were assessed with the inclusion and exclusion criteria. Two reviewers performed this process independently and disagreements were solved by discussion or consulting a third reviewer.

Data Extraction

A standard data extraction form was applied. Data that were extracted consisted of the author, year, study design, sample size, population characteristics, type of disease (AD or PD), type of biomarker (proteomic or genomic), and the type of biomarker measured (Aβ40, Aβ42, a-synuclein total, a-synuclein oligomer, or miRNA), mean values, standard deviations, and total sample sizes in both the experimental and control groups. In case, important data were not available or ambiguous, authors were approached, or missing data were estimated by using available information.

Outcomes Measured

The main outcome measure was the differences in the concentration of salivary biomarkers of AD and PD cases, and healthy controls presented as a mean and standard deviation. This was evaluated in single biomarkers such as Aβ42, Aβ40 in AD; total a-synuclein and oligomeric a-synuclein in PD; and salivary miRNAs in PD. The secondary outcome was the subgroup comparison of disease-specific biomarkers, e.g. Aβ42 vs Aβ40 in AD, a-syn total vs oligomeric a-syn in PD.

Quality and Risk of bias Assessment

The risk of bias assessment of all included observational and case-control studies was assessed according to the Newcastle-Ottawa Scale (NOS), which covered the dimensions of selection, comparability, and outcome. Moreover, to estimate the overall certainty of the evidence on the use of biomarkers as diagnosing tools, the GRADE (Grading of Recommendations Assessment, Development and Evaluation) methodology was used. The heterogeneity and standardization drawbacks were also identified.

Data Synthesis

The Meta Analysis Online tool was used to conduct meta-analysis [13]. A random-effects model was used because of the difference among studies. The odds ratios (OR) and standardized mean difference (SMD) with a 95% confidence interval (CI) was the main effect size. Heterogeneity was assessed based on I2 statistics and I2 >75 % representing significant heterogeneity. Where two or more similar studies were identified, a forest plot was generated against each biomarker outcome. Subgroup analyses were done to compare subtypes of biomarkers (e.g. Aβ42 vs. Aβ40, a-synuclein oligomer vs. total) within disease groups. To examine the stability of the results, sensitivity analyses were performed by decreasing the number of studies that have the largest contributions to heterogeneity. There were 11 studies identified inclusive of 9 case-control, and 2 cross-sectional studies representing both AD and PD populations[14,15,16,17,18,19,20,21,22,23,24].

RESULTS

A total of 222 records were obtained in the initial search. Following the elimination of 130 duplicated studies, 92 studies were determined as eligible by their titles and abstracts. After that, 30 studies were removed because their abstracts were irrelevant, or the information was not comprehensive. The full-texts of 62 studies were evaluated, although 21 studies were removed due to lack of the assessment of a-syn or other biomarkers in both Alzheimer and Parkinson, or inadequate data on outcomes. Eventually, 11 articles were used in the meta-analysis (PubMed = 7, Scopus = 1, Web of Science = 1, Google Scholar = 2). Figure 1 shows the PRISMA workflow.

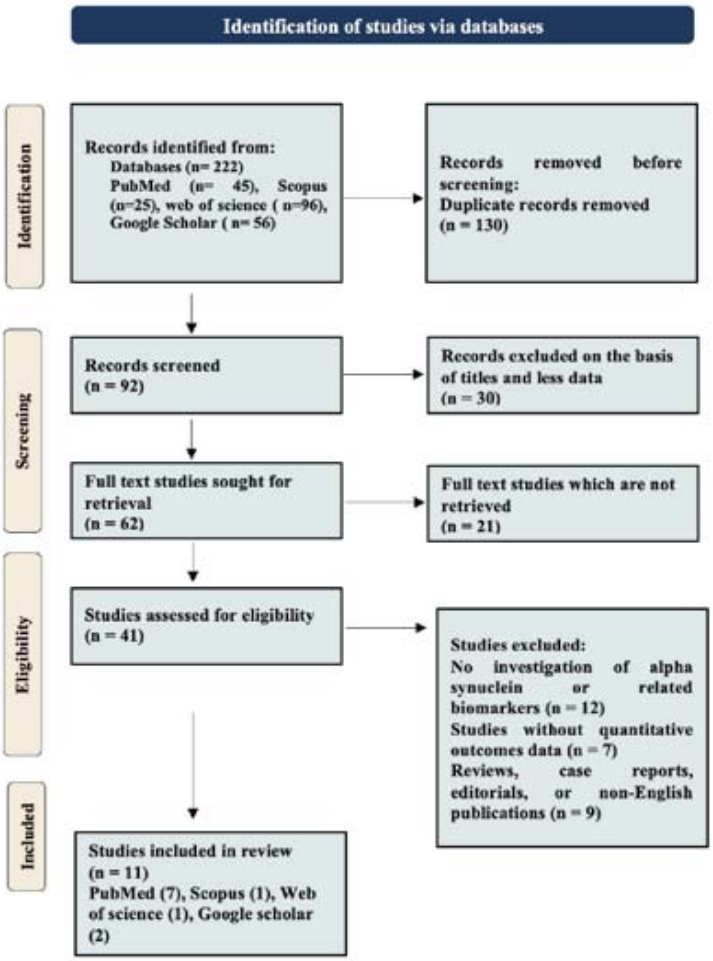

Figure 1: PRISMA workflow diagram

Characteristics of studies

This meta-analysis comprised of 11 studies published in the period of 2016 and 2023, and used a case-control or cross-sectional study design. The research was carried out in a variety of geographical locations such as Europe, Asia, and North America. The size of samples varied between 40 and 202 participants, including patients with the diagnosis of Alzheimer disease (AD), Parkinson disease (PD), or both, and age- and sex-matched healthy participants. The studies included assessed diverse salivary biomarkers, which are mainly categorized into proteomic (e.g., Aβ40, Aβ42, total tau, phosphorylated tau, and total or oligomeric α-synuclein) and genomic (microRNAs (miRNAs), especially miR-153, miR-223, miR-29a-3p, and miR-29c-3p). Proteomic biomarkers were analyzed by the ELISA approach and miRNA profiled by RT-qPCR in most of the studies. There were studies aimed at distinguishing between AD and PD, and others directed at biomarker-based early detection in a single disease group. This heterogeneity in the type of biomarkers, methods of diagnosis, and the disease of interest led to an overall assessment of salivary biomarkers as possible non-invasive biomarkers of early detection of neurodegenerative diseases. **Table 1** illustrates the characteristics of included studies.

Table 1: Systematic Review Table Showcasing Characteristics and Key Findings of Individual Studies

Author (Year)	Study Design	Population Group	Disease Group	Primary Outcome (Diagnostic Accuracy)	Biomarker Type	Biomarkers Assessed
Sabaei et al. 2023 [14]	Cross-sectional	24 AD patients, 24 PD patients, 22 healthy controls (n=70)	AD & PD	AD: Aβ: Sens 62.5%, Spec 91%, AUC 0.81 p-tau: Sens 91.7%, Spec 63.6%, AUC 0.78 α-syn: Sens 66.7%, Spec 68.2%, AUC 0.71 PD: Aβ: Sens 91.7%, Spec 59.1%, AUC 0.77 α-syn: Sens 95.8%, Spec 36.4%, AUC 0.68	Proteomic	Aβ1–42, Phosphorylated tau (p-tau), Total α-synuclein
Ryu et al. 2023 [15]	Case-Control	27 AD patients, 13 healthy controls	AD	AUC (AD vs HC): 0.8946 Sensitivity: 74.07% Specificity: 92.31% AUC (Aβ-PET+ vs Aβ-PET−): 0.9217 Sensitivity: 86.36% Specificity: 88.89%	Genomic (miRNA)	miR-485-3p
Cao et al. 2023 [16]	Case-Control	74 PD patients, 60 healthy controls	PD	α-synOlig: Sens 92%, Spec 86%, AUC 0.941 α-synOlig/α-synTotal: Sens 81%, Spec 71%, AUC 0.772	Proteomic	α-synOlig, α-synTotal, α-synPS129, α-synOlig/α-synTotal ratio
Chen et al. 2022 [17]	Case-Control	30 PD patients, 30 healthy controls	PD	miR-874: Sens 64.3%, Spec 78.6%, AUC 0.727 miR-145-3p: Sens 60%, Spec 75%, AUC 0.707	Genomic (miRNA)	miR-874, miR-145-3p
Jiang et al. 2022 [18]	Case-Control (Multiphasic)	50 PD, 20 ET, 20 MSA, 30 HCs	PD, MSA, ET	Combined miR-29a-3p & miR-29c-3p: AUC = 0.773, Sens = 66.7%, Spec = 83.8% (vs. HCs)	Genomic (Salivary miRNA)	miR-29a-3p, miR-29c-3p, miR-6756-5p
Cui et al. 2022 [19]	Case-control	30 AD patients; 30 healthy controls; plus 15 healthy for method optimization	AD	Aβ42 higher in AD vs controls (most accurately in UPS collection); AUC of 84.83% for Aβ42 alone; AUC improved to 92.11% when combining Aβ42,	Proteomic (Saliva)	Aβ40, Aβ42, t-tau, p-tau; ratios Aβ42/Aβ40, p-tau/t-tau

Shaheen et al. 2020 [20]	Case-control	25 PD patients; 15 healthy age- and sex-matched controls	PD	A-syn total (Sensitivity: 80%, Specificity: 86.7%, Accuracy: 82.3%); A-syn oligomer (Sensitivity: 76%, Specificity: 60%, Accuracy: 72.4%)	Proteomic (Saliva)	Total α-synuclein, Oligomeric α-synuclein
Tvarijonaviciute et al. 2020 [21]	Cross-sectional	69 AD patients (various stages); 83 healthy controls	AD	Complement C4: OR=1.43 (univariate), OR=1.28 (multivariate), AUC=0.613 (p=0.111); Aβ42: OR=0.53 (p=0.041)	Proteomic (Saliva)	Aβ42, Aβ40, t-tau, p-tau181, Complement C4, CRP, PEDF, SAP, α1-antitrypsin, Hp, ADA, FRAP, MIP-4, Cholinesterase
Vivacqua et al. 2019 [22]	Case-Control	100 PD patients, 22 PSP patients, 80 healthy controls	PD	Total α-syn: Sens 67.4%, Spec 91.0% Oligomeric α-syn: Sens 57.0%, Spec 83.9% Oligomeric/Total ratio: Sens 69.8%, Spec 95.2% PD vs PSP (total α-syn): Sens 100%, Spec 96.5%	Proteomic	Total α-synuclein, oligomeric α-synuclein, oligomeric/total α-syn ratio
Cressatti et al. 2019 [23]	Case-Control	83 PD patients, 77 healthy controls	PD	miR-153: Sens 81.8%, Spec 71.4%, AUC 0.79 miR-223: Sens 72.7%, Spec 71.4%, AUC 0.77	Genomic (miRNA)	miR-153, miR-223
Vivacqua et al. 2016 [24]	Case-control	60 PD patients; 40 healthy controls (age- and sex-matched)	PD	Salivary α-syn total significantly lower in PD (5.08 ± 3.01 pg/mL) vs controls (31.3 ± 22.4 pg/mL, p<0.01); α-syn oligomer significantly higher in PD (1.06 ± 0.26 ng/mL) vs controls (0.50 ± 0.20 ng/mL, p<0.01)	Proteomic (Saliva)	α-syn total, α-syn oligomer, α-syn oligomer/total ratio

***AD**: Alzheimer's Disease; **PD**: Parkinson's Disease; **Aβ**: Amyloid-β; **p-tau**: Phosphorylated tau; **α-syn**: alpha-synuclein; **AUC**: Area Under the Curve; **miRNA**: MicroRNA; **PSP**: Progressive Supranuclear Palsy; **ET**: Essential Tremor; **MSA** : Multiple System Atrophy; **CRP** : C-reactive Protein; **PEDF** : Pigment Epithelium-Derived Factor; **SAP**: Serum Amyloid P; **Hp**: Haptoglobin; **ADA**: Adenosine Deaminase; **FRAP**: Ferric Reducing Ability of Plasma; **MIP-4**: Macrophage Inflammatory Protein 4*

Outcomes measures

The primary outcome of this meta-analysis were aimed at analyzing the diagnostic performance of diverse salivary biomarkers in distinguishing Alzheimer s Disease (AD) and Parkinson s Disease (PD) among healthy controls. The results were determined by the quantitative level of expression of certain biomarkers, including Aβ42 and Aβ40 for AD, α-syn total and α-syn oligomer for PD, and microRNAs (miR-153, miR-223, miR-29a-3p, and miR-29c-3p) for PD. These biomarkers were examined to provide their mean differences in disease groups and control with the aim of understanding their diagnostic value. The proteomic and genomic biomarkers

(Aβ and α-synuclein; miRNAs, respectively) were all systematically represented. Nevertheless, because of the diversity of biomarker types in the studies, the comparison was conducted within the biomarker type and not between biomarker types. Other studies also investigated secondary outcomes, i.e. correlation with disease severity (e.g. UPDRS, MoCA, MMSE) or disease duration, however most failed to demonstrate significant correlations with clinical variables. The values of sensitivity, specificity, and AUC were reported in separate studies, yet it was not possible to combine these results because of incompatible reporting forms. Hence, the meta-analysis mainly considered mean and the mean differences in the value of biomarkers as the primary outcome.

Meta-Analysis

This meta-analysis illustrates forest plots to describe diagnostic accuracy of individual biomarkers in saliva in AD and PD. The effect sizes of the individual studies are presented as squares with horizontal lines along the squares reflecting their 95% confidence intervals where the bigger the square, but the size of the square shows the weight of the study in the analysis. The pooled effect size is indicated as a black diamond whose location relative to the line of no effect shows overall statistical significance. The test of heterogeneity was conducted by the I^2 statistic and chi-square test, which indicated the variability of studies beyond chance. The analysis provides insights on the consistency and reliability of salivary biomarkers that could serve as non-invasive diagnostic tool.

The forest plot was created in order to assess the concentration of salivary amyloid-β 42 (Aβ42) in AD. Two eligible studies were used in this analysis, and they included Aβ42 concentration as a biomarker in diagnostics of AD. The pooled odds ratios obtained was 0.70 (95% CI: 0.41 to 1.19) indicating a negative but insignificant relationship between Aβ42 and AD. Although the direction of the effect is consistent with lower concentrations of Aβ42 in AD, the presence of a moderate degree of heterogeneity (I^2 = 38.1%) suggests that there is significant disparity between the studies. These heterogenic factors could be due to various methods of collecting saliva, characterization of the participants, severity of the disease and assay in the laboratories. The forest plot of salivary levels of Aβ42 among the AD patients is given in figure 2.

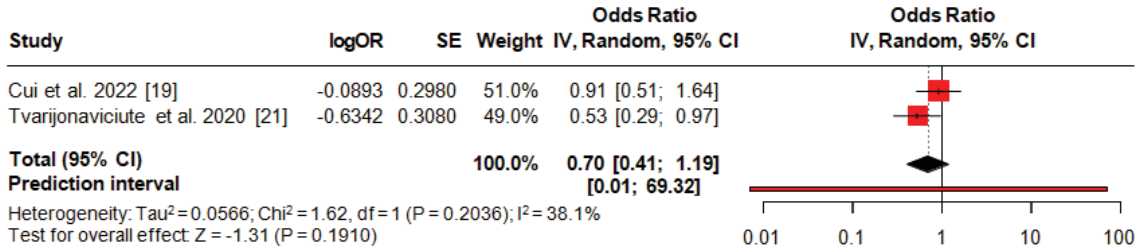

Figure 2: Forest plot of salivary Aβ42 concentration in Alzheimer's disease patients.

The sample size included 99 patients with AD. The findings indicate that Aβ42 could have potential value as non-invasive salivary biomarker of AD, but the evidence is still limited demonstrating the necessity of the adoption of standards and additional large-scale validation studies.

The salivary Aβ40 concentrations, as non-invasive salivary biomarker of AD were analyzed based on the data demonstrated in two studies that met the requirements. The studies showed a slightly higher concentration of Aβ40 in diseased patients with AD, yet the differences in the collection techniques, the sensitivity of the assays, and the characters of the populations led to no heterogeneity. The forest plot of this analysis is demonstrated in **Figure 3**.

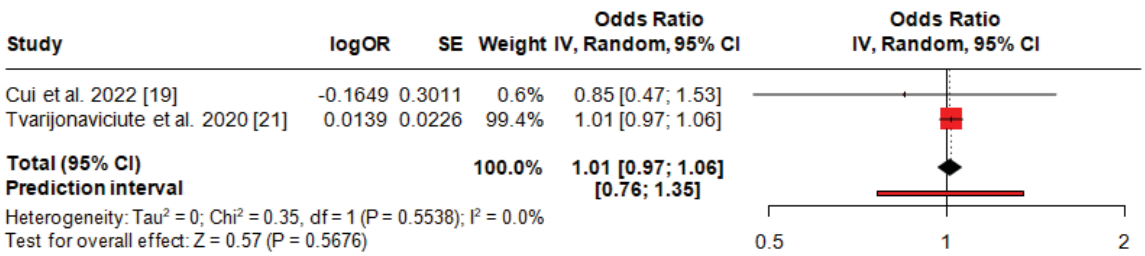

Figure 3: Forest plot of salivary Aβ40 concentrations in Alzheimer's disease participants.

The total sample of the combined analysis was 99 with two studies. The pooled odds ratio of Aβ40 was 1.01 (95% CI: 0.97 to 1.06), which was not significantly different in patients with AD. There was however, no heterogeneity (I^2 = 0%) which indicates strong consistency between studies. Nonetheless, the overall trend of outcomes in both articles indicates that salivary Aβ40 can be used as a supportive tool in the diagnostic process but can hardly be employed as an independent marker.

In Parkinson disease (PD), the analysis of salivary α-synuclein oligomer (α-synOlig) involved four studies evaluating their diagnostic potential. The α-synOlig levels were higher in PD patients than in healthy controls in all the studies indicating that it plays a role in the pathology of PD. Nonetheless, a considerable level of heterogeneity was found, which can be explained by the differences in the processing of saliva (whole vs. exosome), populations, and methodologies. Figure 4 presents the summary of these findings.

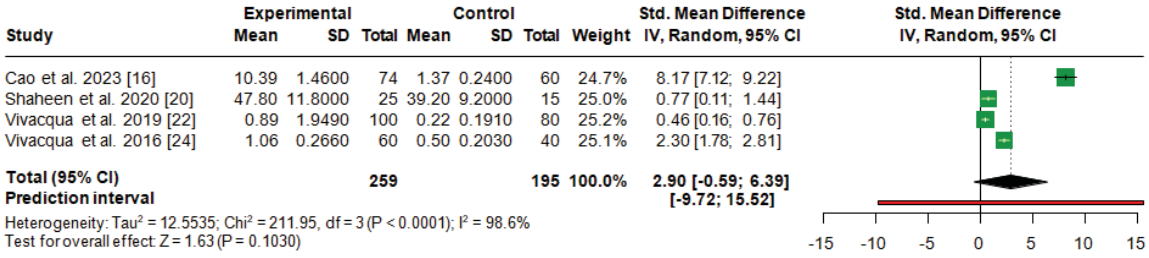

Figure 4: Forest plot of salivary α-synuclein oligomer levels in PD versus healthy controls.

The analysis of 259 PD patients and 195 controls was conducted. Polled standardized mean difference (SMD) was 2.90 (95% CI: -0.59 to 6.39), which revealed the trend of higher α-synOlig in PD, but not statistically significant, since the confidence intervals were wide. The heterogeneity was very high (I^2 = 98.6%), implying high variation among the studies. Nevertheless, the overall direction of effect was consistent across the studies, with PD patients having elevated α-synOlig levels in comparison with controls.

The salivary α-synuclein total (α-synTotal) analysis in PD included four studies that compared PD patients with healthy controls. The results were inconsistent with the overall increasing α-synTotal levels in PD of some studies, and the decreased levels in another. This discrepancy is probably an indication of methodological disparity, including biomarker isolation (whole saliva vs. exosomes) and assay variance. Figure 5 demonstrates overall findings.

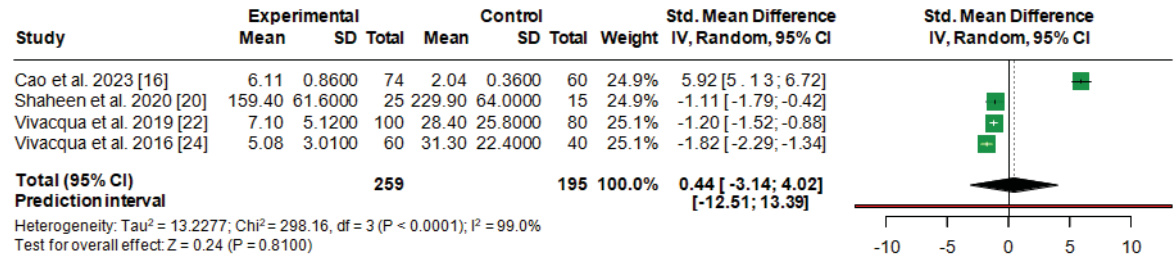

Figure 5: Forest plot of salivary α-synuclein total levels in PD versus healthy controls.

There were a total of 259 PD participants and 195 controls that were evaluated. The random-effects standardized pooled mean difference (SMD) was 0.44 (95% CI: -3.14 to 4.02) showing no significant difference in synTotal between PD and control groups. The heterogeneity was very high (I^2 = 99.0%), which indicates a high level of diversity in study design, populations and methods of analysis. Although the results were mixed, this inconsistency constrains conclusions on the value of α-synTotal as a single diagnostics biomarker in PD.

The expression of salivary miRNA was assessed as a possible genomic marker of PD diagnosis. Although Ryu et al (2023) has also examined miRNA levels in Alzheimer's disease, but we couldn't conduct the analysis because of only one study. Similarly, Jiang et al (2022) and Sabaei et al (2023) also show miRNA in AD and AD & PD, respectively but lack the statistical values. It was added in this study due to its strong systematic knowledge. In contrast to proteomic biomarkers, miRNAs are gene regulatory molecules that might indicate

disease-specific transcriptional modifications. A total of two studies evaluating various miRNAs were retrieved; the miRNAs used were not the same but were within the same diagnostic role in PD since they worked under the same diagnostic section salivary miRNAs. **Figure 6** shows the levels of miRNA in healthy controls and PD patients.

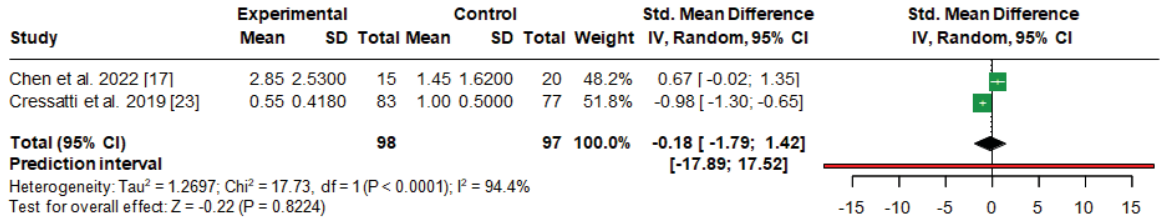

Figure 6: Forest plot of salivary miRNA levels in PD versus healthy controls.

The pooled meta-analysis consisted of 98 PD patients and 97 controls. The standardized mean difference (SMD) was -0.18 (95% CI: -1.79 to 1.42) and the overall miRNA expression showed no significant difference between the PD patients and the controls. The heterogeneity was great (I^2 = 94.40) in spite of the little number of studies, which indicated variability in the chosen miRNA targets, assay approaches, and arguably population characteristics. Although miRNA-based diagnostics in PD had positive effects in one direction with many significant effects and in the other direction, the current evidence is not enough to generalize its use in PD.

The secondary outcome was the comparison of genomic and proteomic biomarkers during the diagnosis of Alzheimer and Parkinson diseases. Since the type of biomarkers would vary in different studies, subgroup comparison was restricted. The analysis, however, evaluated that the proteomic biomarkers had a relatively consistent diagnostic performance than the genomic (miRNA) markers.

Subgroup analysis

Table 2: Subgroup Analysis of Amyloid-Β Biomarkers in Alzheimer's disease and A-Synuclein Biomarkers in Parkinson's disease

Biomarker Subgroup	Disease	Studies (n)	Pooled effect measures	95% CI	Heterogeneity (I^2)	Interpretation
Aβ42	AD	2	OR = 0.70	(0.41, 1.19)	38.1%	Moderate heterogeneity; Aβ42 levels lower in AD; potential diagnostic value but not conclusive.
Aβ40	AD	2	OR = 1.01	(0.97, 1.06)	0.0%	No significant difference; highly consistent results; limited diagnostic value for AD.
α-syn Oligomer	PD	4	SMD = 2.90	(-0.59, 6.39)	98.6%	Substantial effect but extremely high I^2 limits reliability; supports elevation in PD.
α-syn Total	PD	4	SMD = 0.44	(-3.14, 4.02)	99.0%	Very high inconsistency; total α-syn less reliable than oligomer

Aβ: Amyloid β; α-syn: α-synuclein; AD: Alzheimer's disease; PD: Parkinson's disease; CI: confidence Interval; α-synOligo: α-synuclein Oligomer; OR: Odds ratios; SMD: Standard mean difference.

There were two groups of biomarkers measured in the subgroup analysis: Aβ40 and Aβ42 in AD, and α-synuclein oligomer and α-synuclein total in PD. Within the AD subgroup, Aβ42 demonstrated a better effect direction but with moderate heterogeneity compared to Aβ40, indicating that Aβ42 indicates a more reliable biomarker than the other. On the other hand, in the PD subgroup, the α-synOligo showed a higher pooled effect size compared to total α-synuclein, suggesting that oligomeric forms might be more strongly associated with PD pathology. However, the heterogeneity presented in both PD biomarkers was large, indicating that the included studies differed significantly in the nature of the samples, techniques, and disease progressions. A subgroup analysis of various biomarkers is demonstrated in **Table 2.**

The subgroup analysis overall indicates that oligomers (in α-synuclein) and Aβ42 have the potential to be more diagnostically accurate than the α-synodal and Aβ40, but more uniform studies are required to prove these tendencies.

Table 3: Sensitivity Analysis Results across Different Biomarker Groups

Biomarker Group	Sensitivity Analysis Performed	Effect on Pooled Result	Effect on Heterogeneity (I^2)	Remarks
Aβ42 in AD	No study removed	Pooled OR remained stable (0.70; 95% CI: 0.41–1.19)	**Moderate (38.1%)**	Pooled effect remains stable; moderate heterogeneity likely due to sampling methods and assay variability.
Aβ40 in AD	No study removed	Stable pooled OR (1.01; 95% CI: 0.97–1.06)	**0% (No heterogeneity)**	Results consistent between both studies; robust and reliable outcome.
α-synuclein Oligomer in PD	Removed **Cao et al. (2023)**	Pooled SMD slightly reduced (**from 2.90 to 1.17**)	I^2 reduced slightly (**98.6% → ~94.5%**)	Removal improved heterogeneity marginally.
α-synuclein Total in PD	Removed **Cao et al. (2023)**	Pooled SMD reduced in value (**SMD dropped from 0.44 to ~ -1.39**)	I^2 reduced from **99.0% to ~59.8%**	Cao's sample type (whole saliva) contributed to major heterogeneity; removal improved stability.
miRNA in PD	Removed **Cressatti et al. (2019)**	Direction shifted (**from negative to slight positive but NS**)	I^2 reduced significantly (**94.4% → ~38%**)	Heterogeneity primarily driven by different miRNA targets across studies (miR-153 vs miR-874).

Aβ: Amyloid Beta; miRNA: microRNA; OR: Odds ratios; SMD: standard mean difference.

Sensitivity analysis

A sensitivity analysis was performed to determine how robust the meta-analytic findings were by systematically removing the single studies and determining its impact to the overall pooled effect. The procedure assists in identifying whether one or more studies exerted a disproportionate influence on heterogeneity or overall outcome. Particularly, the elimination of Cao et al. (2023) in the study of α-synOligo in PD slightly reduces heterogeneity from 2.90 to 2.27. Similarly, the exclusion of Cao et al. (2023) in the analysis of α-synuclein total in PD significantly decreases heterogeneity, making it a possible source of heterogeneity. Likewise, the omission of Cressatti et al. (2019) changed the direction of the effect in the miRNA subgroup, indicating a considerable impact caused by the different miRNA types measured. The sensitivity analysis on Amyloid-β (Aβ42 and Aβ40) biomarkers in AD expressed the pooled estimate to be stable and there was little change, indicating no variation throughout the studies. The sensitivity analysis of various biomarkers is indicated in **Table 3**.

In general, the sensitivity analysis has validated the fact that although certain heterogeneity was observed especially in the α-synuclein and miRNA subgroups, overall the results were still largely consistent.

Risk of Bias Assessment

Table 4: Risk of bias assessment of observational studies using the Newcastle-Ottawa Scale (NOS).

Study	Selection (max 4)	Comparability (max 2)	Outcome/Exposure (max 3)	Total Score (max 9)
Sabaei et al. (2023) [14]	★★★★	★★	★★★	9
Ryu et al. (2023) [15]	★★★	★	★★	6
Cao et al. (2023) [16]	★★★★	★★	★★	8
Chen et al. (2022) [17]	★★★	★	★★	6
Jiang et al. (2022) [18]	★★★	★	★★	6
Cui et al. (2022) [19]	★★★★	★★	★★	8
Shaheen et al. (2020) [20]	★★★	★	★★	6
Tvarijonaviciute et al. (2020)[21]	★★★★	★★	★★	8
Vivacqua et al. (2019) [22]	★★★	★	★★	6
Cressatti et al. (2019) [23]	★★★★	★★	★★	8
Vivacqua et al. (2016) [24]	★★★	★	★★	6

Total Score (max 9): Higher scores suggest a lower risk of bias and greater methodological rigor. 7–9 stars: Low risk of bias, 4–6: Moderate risk of bias, <4: High risk of bias

The studies included were heterogeneous in design, the size of the population, and the methods used in assessing outcomes. The Newcastle-Ottawa Scale (NOS) of observational studies was used to assess risk of bias. A majority of the studies were either moderate or high in quality, with clearly outlined selection processes and comparability and blinding were low. **Table 4** presents the detailed risk of bias evaluation of all the studies that were included.

The certainty of evidence was moderate to low, primarily because of the scarcity of genomic evidence and wide heterogeneity among trials. The relative certainty of proteomic biomarkers was relatively moderate according to consistent results.

DISCUSSION

This meta-analysis systematically designed salivary proteomic and genomic biomarkers as potential, non-invasive biomarkers of early detection of Alzheimer Disease (AD) and Parkinson Disease (PD). Our main findings proved substantial diagnostic value of proteomic biomarkers of Aβ42 and Aβ40 in AD and a-synuclein oligomers and total a-synuclein in PD. Besides, the research also included genomic biomarkers, namely salivary miRNAs, although they varied among the studies (miR-485-3p in AD in Ryu et al (2023) and miR-153/miR-29 family in PD). However, they were pooled in the area of generalized genomic biomarker to indicate the extended diagnostic potential of salivary miRNA signatures in neurodegenerative disorders especially in the analysis of PD. The strategy outlines a valuable outcome in the consideration of saliva as a biofluid in non-invasive diagnostics of neurodegeneration.

The findings showed that across the amyloid biomarkers, Aβ42 showed a more significant discriminative power in AD than Aβ40, which is consistent with its established role in the pathogenesis of AD. In PD, the level of a-synOligo was significantly higher, and the level of a-synTotal was correlated lower in both cases acting as appropriate diagnostic biomarkers in accordance with the mechanisms of neurodegenerative diseases. The analysis of genomic biomarkers itself was quite substantial, but it demonstrated a divergent nature. The two miRNA studies included in the meta-analysis did not use the same miRNAs or disease target, which is a significant limitation, but both showed a very strong difference between cases and controls supporting the potential but highlighting the gap of miRNA biomarker research in PD.

Our findings on salivary Aβ biomarkers in AD coincide with previous studies that highlight the diagnostic value of Aβ42 compared to the Aβ40 [25,26]. Several studies have found that salivary Aβ42 concentration tends to be higher or has higher distinction between patients with AD and healthy individuals compared to Aβ40, which tends to lack sufficient diagnostic accuracy as a result of its more widespread distribution in disease patients and healthy subjects [27]. Similar patterns were identified in studies assessing cerebrospinal fluid (CSF) and plasma biomarkers, indicating that Aβ42 has disease specificity in biofluids [28,29]. The heterogeneity of the diagnostic performance of Aβ40, as expressed in our forest plot, however, reflects the inconsistencies already observed in plasma studies, where Aβ40 has

not been shown to have the same discriminative power that Aβ42 has in detecting AD [30]. This highlights similar biological properties of these biomarkers regardless of the types of samples and further supports particularity of Aβ42 to AD pathology [31].

The findings concerning the a-synuclein biomarkers in PD also support previous data [32]. Many studies have confirmed that levels of a-synuclein oligomers are always increased in PD, whereas a-synuclein total levels are often reduced, probably because of aggregation and depletion out of soluble pools [33,34]. This tendency is not only described in saliva but also in cerebrospinal fluid and blood-based exosomes [35]. This biological model is consistent with our results, but the total a-synuclein heterogeneity indicates that this variability may be caused by factors such as disease stage, saliva collection methods, and detection methods [36]. Furthermore, the presence of miRNA dysregulation in PD is also supported by literature, with our genomic biomarker profile being consistent with other published reports; yet, there is no consensus regarding the choice of miRNAs used in PD miRNA biomarker research [37,38]. This microRNA target dissimilarity indicates a need of harmonization in biomarker discovery process [39,40].
Although it makes important contributions, this meta-analysis has several limitations. The greatest difficulty was the diversity of the types of biomarkers between AD and PD; proteomic markers were disease-specific (Aβ within AD, a-synuclein in PD) without overlap, and genomic biomarkers denoted unique miRNAs based on disease. This eliminated disease-to-disease comparisons and aggregated biomarker analysis. Higher heterogeneity of the specified analyses (especially miRNA and a-synuclein total studies) was contributed by the variability of saliva collection procedures, detection platforms, and demographics of the patients. Moreover, small sample size in some of the studies reduces the applicability of the inferences. Further studies should be of a larger magnitude, in multi-cohorts, standardized saliva processing methodology and a uniform set of biomarkers to be studied. Combining the multi-omics (proteomics, genomics, and metabolomics) and machine learning models may significantly increase the accuracy of diagnosis. In addition, there is need to conduct longitudinal studies to establish whether salivary biomarkers can also predict disease in addition to diagnosis, treatment response, and prognosis.

CONCLUSION

This meta-analysis reveals that salivary proteomic and genomic biomarkers have high potential to be applied as non-invasive diagnostic tools for early diagnosis of Alzheimer and Parkinson disease. There was an increased diagnostic value of proteomic biomarkers, such as Aβ42 and a-synuclein oligomers and a heterogeneous finding of the genomic biomarkers because of the inconsistency targets of miRNA. Although the results are encouraging, direct comparisons between AD and PD are constrained by the absence of universal biomarkers around studies. Additional studies of large sample size and standardization are required to validate these biomarkers in the existing clinical practice.

LIST OF ABBREVIATIONS

AD Alzheimer 's disease
PD Parkinson 's disease
Aβ Amyloid Beta
a-synOligo alpha-Synuclein Oligomers
a-synTotal alpha synuclein Total
miRNA MicroRNA

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None

CONFLICT OF INTEREST

None

AUTHORS' CONTRIBUTION

All contributed equally as per ICMJE.

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